Vol. XL May, 1951 No. 5

SCIENTIFIC EDITION

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

Justin L. Powers, Editor

Committee on Publications

George D. Beal, *Chairman*
B. V. Christensen
Robert P. Fischelis
Don E. Francke
Hugo H. Schaefer

Editorial Advisory Board

L. W. Busse
John E. Christian
T. C. Daniels
L. W. Hazleton
E. J. Hughes
H. W. Youngken, Jr.

CONTENTS OF THIS ISSUE

Arabic Acid and Its Salts. II. The Effect of Cations on the Viscosity of Arabic Acid Solutions.... 221
R. H. Schleif, T. Higuchi, and *L. W. Busse*

A Simple Aerosolizing Apparatus for the Production of Experimental Asthma..... 226
Joseph W. E. Harrisson, Julian L. Ambrus, and *Clara M. Ambrus*

Studies on *Mahonia* Genus. IV. *M. Leschenaultii* (Wall.) Takeda, *M. Manipurensis* Takeda, and *M. Sikkimensis* Takeda..... 229
R. Chatterjee and *M. P. Guha*

Studies on *Mahonia* Genus. V. Structure of Neprotine..... 233
R. Chatterjee and *M. P. Guha*

The Ultraviolet Determination of Aureomycin and Terramycin..... 237
Dorothy J. Hiscox

Terramycin: Chemical Methods of Assay and Identification..... 241
F. Monastero, John A. Means, T. C. Grenfell, and *F. Howard Hedger*

The Determination of Choline in the Presence of B Complex Vitamins..... 245
F. J. Bandelin and *J. V. Tuschhoff*

A New Mercurial Diuretic..... 249
E. Brown Robbins and *K. K. Chen*

A Note on the Preparation of the Tocopherol Blank in the Fox and Mueller Procedure for Vitamin A 252
D. G. Chapman and *J. A. Campbell*

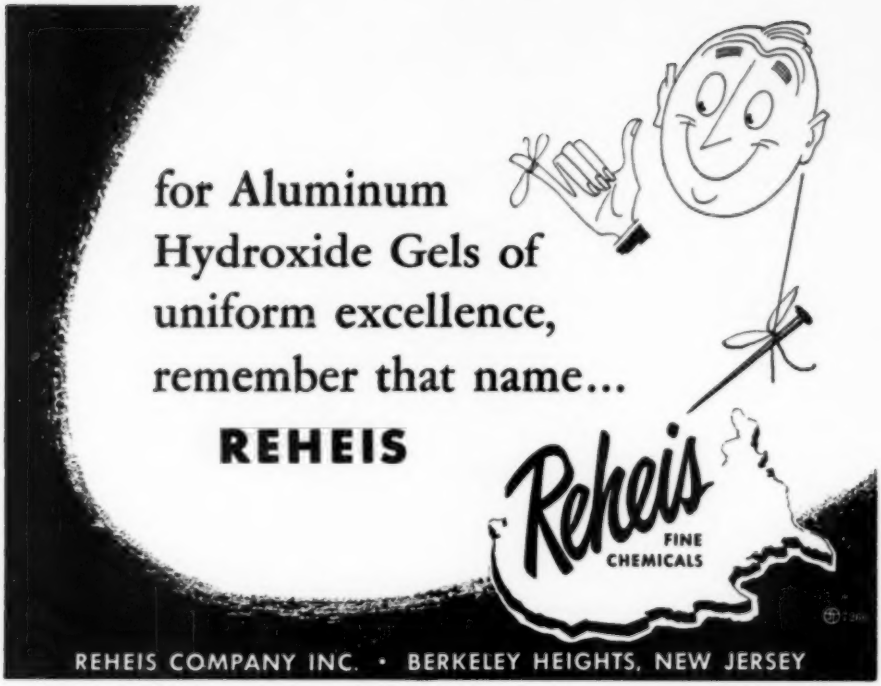
A Note on the Alkaloid Content of *Gloriosa Rothchildiana*, O'Brien..... 253
J. T. Bryan and *W. M. Lauter*

Preliminary Report of Lysis of Mouse Sarcoma 37 by a Component Isolated from Resin of Podophyllum..... 254
Muriel R. Loran and *Earl P. Guth*

Book Reviews..... 255

Advertising..... Cover pages 2, 3, 4 and pp. I–IV

Copyright, 1951, by the American Pharmaceutical Association



NOTICE

PAPERS FOR SECTION MEETINGS
OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

The next annual meeting of the American Pharmaceutical Association will be held in Buffalo, New York, August 26 to September 1, 1951. Members planning to present papers before any Section during this meeting are earnestly requested and strongly urged to notify the appropriate Section secretary of their intention promptly. Titles and abstracts (not to exceed 250 words) of papers should be submitted by June 1, 1951 or preferably earlier, in order to insure a place on any Section program. Send titles and abstracts of papers to:

SECTION SECRETARIES

SCIENTIFIC
RAY S. KELLEY, Secretary
179 Longwood Avenue
Boston 15, Mass.

PHARMACEUTICAL ECONOMICS
FRANCIS J. O'BRIEN, Secretary
Albany College of Pharmacy
Albany, New York

EDUCATION AND LEGISLATION
JOHN L. VOIGT, Secretary
University of Mississippi
School of Pharmacy
Oxford, Miss.

PRACTICAL PHARMACY
ELMER M. PLEIN, Secretary
University of Washington
College of Pharmacy
Seattle 5, Wash.

HISTORICAL PHARMACY
EDWARD S. BRADY, Secretary
1436 W. 48th Street
Los Angeles 37, Calif.

Handsome Durable Binders for Journals of the American Pharmaceutical Association

Combination Sets for Practical Pharmacy and Scientific Editions $5.50 a pair
For Single Editions $3.25 each

These binders open flat as a bound book. Made of durable imitation leather, they will preserve your journals permanently. Each cover holds 12 issues (one volume)....Do your own binding. Instructions easy to follow. Mail coupon for binders on 10 day trial.

.....**Mail Coupon Today**.....

SUCKERT LOOSE-LEAF COVER CO.
234 W. LARNED ST. DETROIT 26, MICH.

Mail postpaid....binders for the Journal of the American Pharmaceutical Association ☐ Practical Pharmacy Edition ☐ Scientific Edition for years.....
....Will remit in 10 days or return binders collect.

Name.....

Address.....

City.....State.....

LIST OF MISCELLANEOUS REPRINTS

Interesting and Instructive Pharmaceutical Articles
(15¢ each)

Action of Drugs on Parasympathetic Nervous System, by M. W. Green
A. PH. A. Official Roster and Pharmaceutical Directory
Are Animal Experiments Needed? by A. C. Ivy and A. F. Zobel
Clinical Toxicity of Thiouracil
Suggested Plans for Hospital Pharmacies
Practical Guides to New F.D.A. Labeling Regulation
Proposed Uniform State Pharmacy Act
The Relation of the Pharmacopeia to the Pharmaceutical Profession
Some Modified Formulas for Dermatotherapy
Survey of Pharmacy Laws, by P. A. Costello

Pictures
($1 each; $1.50 any 2)

Frederick B. Power
Pharmakeutike—Sculptural Panel
Phos-Kai-Elpis—Sculptural Panel
The Laboratory *(a famous misnamed picture)*

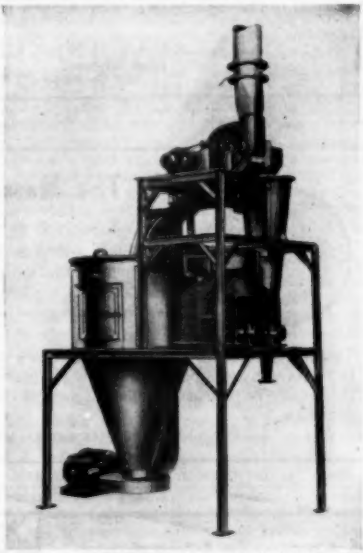
Miscellaneous Items

A. PH. A. Code of Ethics—In two colors, sizes 12 x 18" or 10 x 13½", 25¢
Badianus Manuscript—color plate reprint, 50¢
The Oath and Prayer of Maimonides, (2 colors), 25¢
Table of Metric Doses, 1½¢ each, 100 for $1.25

Now...

A SPRAY DRYER THAT MEETS COST AND PRODUCTION LIMITATIONS OF THE SMALLER COMPANY!

★ This Bowen unit is largely preassembled before shipment to simplify installation and minimize costs. It requires a floor space of only 10' x 15'. Capacity range is two to seven tons of product per 24 hour period depending upon the characteristics of the material. Actual output can be determined by a simple test run in our laboratory.



Bowen Model 2 Spray Dryer

TEST SERVICES

Bowen laboratories are equipped to spray dry samples as small as one gallon for preliminary study, or to produce sizeable quantities of material for market sampling.

BOWEN
ENGINEERING, INC.

NORTH BRANCH 7
NEW JERSEY

Recognized Leader in Spray Dryer Engineering ...Since 1926

SPRAY DRYERS • EVAPORATORS • DUST COLLECTORS

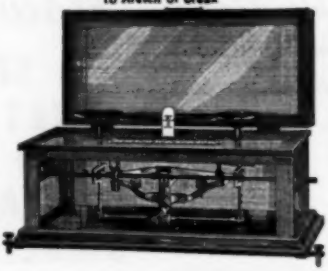
Scale illustrated is our
No. 540 all Glass Model

Capacity—4 oz. (120 grams)
Sensitivity—1/10 grain
Finish—Genuine light mahogany
with chrome plated parts
Price—$135.00 (subject to
change without notice)

PROMPT DELIVERY
on this as well as other class A scales

No. W35 (all glass in lid).....$100.00
No. W33 .....90.00
All With Stainless Steel Pans

Our scales have no bands or wires to stretch or break



High quality
Prescription
Scale made by
HENRY TROEMNER
Manufacturer of the Finest Scales for the Druggist Since 1840
911 Arch Street
Philadelphia 7, Pa.
(*Send for Circular A-A*)

COLLEGE OF PHARMACY AND CHEMISTRY

A co-educational institution offering degree courses in Pharmacy (B.S. Pharm.) and Chemistry (B.S. Chem.). Graduation from an accredited high school or the equivalent is required for admission.

Because the College is located in the pharmaceutical manufacturing center, ample opportunities are open to the students to become acquainted with the most modern and practical methods. The College, the Alumni, and Detroit pharmacists all cooperate to assist students in securing part-time employment. Write for catalog.

DETROIT INSTITUTE OF TECHNOLOGY
2020 Witherell Detroit 26 Michigan
303 Downtown YMCA - Grand Circus Park

The Massachusetts College of Pharmacy
Longwood Avenue, Boston

Graduate study in Boston has always appealed strongly to students in the field of medicine and public health. The environment is stimulating and attracts students from all parts of the world. Library, laboratory, hospital, and other facilities for graduate work are outstanding in this area.

The Massachusetts College of Pharmacy is located in a great medical and educational center. It offers unusual opportunities to graduates of colleges of pharmacy who are interested in further study in pharmacy, pharmaceutical chemistry, and pharmacognosy. A few fellowships are available.

For additional information, write to HOWARD C. NEWTON, Dean.

A Career in Pharmacy and the Allied Sciences

Philadelphia College of Pharmacy and Science.

Pharmacy, Chemistry, Bacteriology and Biology offer opportunities for interesting and successful careers to young men and women. B.Sc. degree courses. Graduate study leading to M.Sc. and D.Sc. degrees.

Write for catalog to

43rd St., Woodland and Kingsessing Aves., Philadelphia 4, Pa. Founded in 1821.

Published by the American Pharmaceutical Association under supervision of the Committee on Publications with the approval of the Council. Publication office, 20th and Northampton Sts., Easton, Pa. Editorial office: Scientific Edition, 2215 Constitution Ave., Washington 7, D. C.

Annual Subscription—Journal of the American Pharmaceutical Association, complete (both editions): United States and Pan America, $7.00; Canada, $7.70; other foreign, $8.00; members of the American Pharmaceutical Association with dues, $4.00. Each edition, Scientific Edition or Practical Pharmacy Edition: United States and Pan America, $4.00; Canada, $4.35; other foreign, $4.50. Single numbers, either edition: United States and Pan America, $0.35; Canada, $0.40; other foreign, $0.50.

Claims for missing numbers will not be allowed (1) if received more than 60 days from date of issue, (2) if loss was due to failure to give 30 days notice of change of address, or (3) if reason for claim is "missing from files."

Entered as second-class matter January 23, 1917, at the Post Office at Easton, Pa., under the act of March 3, 1879, as 24 times a year; Scientific Edition monthly on the fifth; Practical Pharmacy Edition monthly on the 20th. Acceptance for mailing at a special rate of postage provided for in Section 1103, Act of October 3, 1917, authorized July 10, 1918.

Robert P. Fischelis, *Secretary and General Manager*

NOTICE TO AUTHORS OF PAPERS

Manuscripts.—All manuscripts submitted to the Editor or presented to the Sections of the ASSOCIATION should be ready for publication, typewritten with double spacing on one side of good quality paper 8.5″ x 11″ in size and with 1″ to 1.5″ margins. In addition to the original typewritten copy one or more carbon copies should be submitted in order to facilitate examination by members of the Editorial Advisory Board or other referees. Each manuscript should be accompanied by an abstract of not more than 250 words including a summarization of conclusions and recommendations. All pages in manuscripts, including tables but not figures, should be numbered consecutively. Previously published and readily accessible procedures which have been followed in experimental work should not be made a part of the manuscript, but should only be referred to by appropriate literature references.

Form of Presentation.—Titles of manuscripts should be kept as short as possible and preferably restricted to one line. The name of the laboratory in which the work described was done should be placed in a footnote at the end of the first page. Each manuscript should begin with one or more introductory paragraphs indicating the intention and meaning of the investigation and the method of procedure. The introduction should be followed by a report of the essential details of the experimental work, and a discussion or interpretation where necessary. Finally there should be a brief summary of conclusions and recommendations. Authors may find it helpful to arrange manuscripts to conform to the style of presentation as illustrated in papers on analogous subjects published in recent issues of THIS JOURNAL.

In preparing manuscripts for publication in THIS JOURNAL center headings should be avoided so far as possible. Side headings followed by a period and dash should be indented and run into the part of the text to which they apply. Such side headings may be used as freely as desired. Literature citations should be numbered consecutively in order of appearance in the manuscript and should be indicated by full-sized Arabic numerals enclosed in parentheses. If a literature reference is cited more than once in a manuscript subsequent citations should be indicated by the first number assigned. References to footnotes throughout the text should be numbered consecutively by superscript Arabic numerals, but such references in each table should be designated by superscript lower case letters beginning with "a" and continuing in natural order. Literature citations should be grouped at the end of the manuscript under the heading "References." The citations should be numbered consecutively with full sized Arabic numerals enclosed in parentheses corresponding to the numbers used in the text. The names of all periodicals cited in the list of references must be abbreviated in accordance with abbreviations given by *Chemical Abstracts* in its "List of Periodicals Abstracted." The sequence followed in the citations must be as follows:

(1) Doe, J. B., *Am. J. Physiol.*, **79**, 289 (1927).

References to books should be in the order given, (1) author's surname and initials, (2) title of the book, (3) edition, (4) name of publisher, (5) place of publication, (6) year of publication, (7) volume, and (8) page number. A complete book reference may be illustrated as follows:

(1) Gilman, H., "Organic Chemistry," ed. 2, John Wiley and Sons, New York, 1943, vol. 2, pp. 1236, 1252.

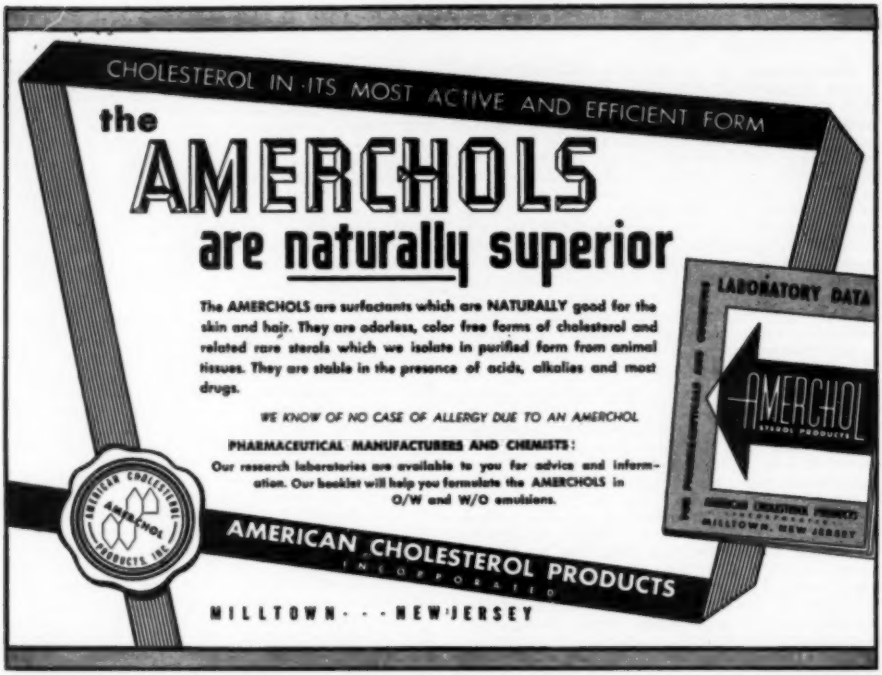
Arabic numerals must be used to designate all definite weights, measurements, percentages and degrees of temperature unless occurring at the beginning of a sentence, in which case the numeral and measurement are spelled out. Periods of time should be written in words. Unusual characters and Greek letters should be written plainly and legibly or should be explained by notes on the margin of the page. Fractions should be written with a slant line. Standard abbreviations should be used whenever weights and measures are stated in the metric system, e.g., 10 Gm., 2.5 cc., etc. The forms to be used are: cc., Kg., Gm., mg., mm. cm., L. and M.

Figures.—All drawings should be made in India ink, preferably on white tracing paper or cloth. Original drawings are preferable, but clear, well-prepared glossy photographic prints are acceptable. The size of illustrations should not exceed the 8.5″ x 11″ manuscript size. They should be designed for reduction to the width of one column (2.75″) or full page width (5.5″). Thus, height rather than width should be accentuated where possible.

Numbers and measurements, etc., which are to appear on the axes of the drawing should be indicated in pencil outside the axes, in order that they may be set in type. Captions and legends should be placed *below* the diagram (not on it), or, if lengthy, collected on a separate typewritten list, identifying each by its proper figure number and including whatever acknowledgments are necessary.

Photographs should never be rolled, and paper clips should not be used unless guarded by a piece of paper folded several times to prevent an indentation in the photograph. All drawings and photographs should be protected from bending and from the chance of having their edges torn while in transit. Do not write on the back of a photograph. Any indentation in the photograph, however slight, will be likely to show as a blemish in the reproduction. All figures submitted must be referred to in the text of the manuscript, and should be numbered consecutively with Arabic numerals, e.g., Fig. 1, Fig. 2, etc. Kymograph tracings should not be submitted with the original experimental legends which are likely to have been hastily or carelessly inscribed. Authors may find it worth while to employ a professional draftsman to make the drawings in proper form for reproduction.

Tables.—Tables should be carefully constructed so that the data presented may be understood easily. The indiscriminate use of tables should be avoided. Tables should be so constructed as to occupy fully a single column (2.75″) which will take about 40–50 characters and spaces. Tables which require two columns are also acceptable. Interpretive statements should not be written on the body of a table but should be explained in footnotes. Column heads should be made as small as possible and standard abbreviations should be used liberally. Data which may easily be deduced by simple arithmetic from data in another column should not be included. All tables should be referred to in the text of the manuscript and the author should indicate as nearly as possible the positions in which the tables should be set. It is not necessary to place tables on separate sheets, and in fact it is preferable to intersperse them in the manuscript. Tables should be numbered consecutively using Roman numerals. The table number and the title should be placed in a continuous heading above the data presented. The preparation of a clear and concise table of data requires much planning and infinite care.


CHOLESTEROL IN ITS MOST ACTIVE AND EFFICIENT FORM
the
AMERCHOLS
are naturally superior
The AMERCHOLS are surfactants which are NATURALLY good for the skin and hair. They are odorless, color free forms of cholesterol and related rare sterols which we isolate in purified form from animal tissues. They are stable in the presence of acids, alkalies and most drugs.
WE KNOW OF NO CASE OF ALLERGY DUE TO AN AMERCHOL
PHARMACEUTICAL MANUFACTURERS AND CHEMISTS:
Our research laboratories are available to you for advice and information. Our booklet will help you formulate the AMERCHOLS in O/W and W/O emulsions.
LABORATORY DATA
AMERCHOL
AMERICAN CHOLESTEROL PRODUCTS INCORPORATED
MILLTOWN · · · NEW JERSEY

WANTED—JOURNALS OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

SCIENTIFIC EDITION

1912: January, March, April, May, June, July. **1913:** May. **1914:** May, June, August, September, October, November, December. **1915:** January, February, March, April, May, June, July, September, October. **1916:** All issues. **1917:** January, March. **1918:** January, February, April, May, July, December. **1919:** January, February, March, April, July, August, October, November. **1920:** January. **1922:** January. **1938:** January. **1940:** January. **1941:** April. **1944:** January, April, May, June, August, November. **1945:** January, February, March, April, June, September. **1946:** January. February, March, April, October, November. **1950:** January.

PRACTICAL PHARMACY EDITION

1940: August, November. **1941:** January. **1945:** April. **1946:** January.

Special offers for these issues will be quoted upon request.
Please ship to the American Pharmaceutical Association, 2215 Constitution Ave., N. W., Washington 7, D. C. Number each package in the shipment consecutively starting with "one" and mark clearly the name and address of the sender. Mail packing lists to the American Pharmaceutical Association.
List of May, 1951 subject to withdrawal without notice.

Scientific Edition

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

JUSTIN L. POWERS, EDITOR, WASHINGTON, D. C.

VOLUME XL — *MAY, 1951* — NUMBER 5 — CONSECUTIVE NO. 10

Arabic Acid and Its Salts

II. The Effect of Cations on the Viscosity of Arabic Acid Solutions*

By R. H. SCHLEIF, T. HIGUCHI, and L. W. BUSSE

Curves illustrating the effect of cations on the viscosity of arabic acid solutions are shown and discussed. The relationship of these data to the stability of emulsions and suspensions is considered.

A PREVIOUS REPORT dealt with the preparation of arabic acid in the powdered form by the spray-drying process (1). In this paper, data are presented which show the influence of several cations on the viscosity of arabic acid solutions. The application of these results to pharmaceutical preparations is discussed.

In order to interpret the curves obtained in the viscosity study, it is first necessary to review briefly the viscosity studies made by Kruyt and Bungenberg de Jong on colloidal solutions (2). It has been noted by these investigators that certain colloidal solutions which contain charged colloidal particles exhibit a peculiar type of curve when the specific viscosity ($\eta_{sp.}$) over concentration (c) was plotted against concentration. This behavior is attributed to the charge on the molecule and is known as the electroviscous effect. The influence of the ionic environment on the viscosity is a phenomenon very typical of solutions of charged, solvated macromolecules. It is clear that in the presence of charges the skein will not assume its statistically most probable form, but will have a less dense form through the interaction of the charged spots (mutual repulsion), or a more compact form if positive and negative charges are present simultaneously. As a result, the viscosity, which is sensitive to the form of the skein, is one of the most important aids in the investigation of systems with charged macromolecules. If there are only charged spots of one sign on the molecule, then the molecule will be relatively spread out. If charged spots of both signs are present, then the molecule will be more compact than corresponds to the most probable state. This remodeling of the skein not only depends on the interaction of the charged spots, but also on the extension of the ion atmosphere around each charged spot. The shape of the molecule will therefore also depend on the concentration and nature of indifferent electrolytes in the solution, since these determine the thickness of the ion atmosphere.

When specific viscosity/concentration is plotted against concentration, Kruyt and Bungenberg de Jong have observed for agar solutions and other colloids that as the concentration is decreased the

* Received October 23, 1950, from the University of Wisconsin, School of Pharmacy, Madison.

curve decreases to a minimum, then at low concentrations a rapid rise occurs again. An example of this type of curve may be found in the gum arabic curve in Fig. 1. The rapid rise shown in the curve has been attributed by these workers to the charge on the molecule which affects the viscosity to a larger extent at low concentrations. They called the viscosity due to the charge the electroviscous effect. It was also noted by them that electrolytes added to the colloidal solutions will suppress this charge, resulting in a curve which is linear. For colloidal solutions with a negative charge the addition of an electrolyte with a divalent cation will result in a greater suppression of the charge, and a trivalent cation would suppress it still further. Suppression of the charge results in a more compact molecule; therefore, some of the water surrounding the molecule is squeezed out, resulting in less hydration and lowered viscosity.

Self-suppression of the charge by the colloidal particles at a higher concentration has been given as the explanation for the minimum in the curve discussed above.

Gum arabic, from which the arabic acid for the investigation was derived, is an example of a colloid with a negative charge. This charge is due to the carboxyl groups in the molecule. It would be expected, therefore, that a charge would exist on molecules of arabic acid and its salts as it does on gum arabic.

EXPERIMENTAL

The viscosity studies were made with a Fenske viscosimeter in a constant temperature bath set at 30°. The specific gravity of each solution was determined with a Westphal balance. All solutions were filtered with a sintered glass funnel before determinations were made.

Determinations were made both with and without the presence of 0.5 *M* sodium chloride and 0.5 *M* calcium chloride.

The solutions for the determinations were prepared as follows:

Sodium Arabate.—Prepared or discussed previously (1).

Potassium Arabate.—A solution (20%) of arabic acid was neutralized to *p*H 7 with 0.5 *N* potassium hydroxide.

Calcium Arabate.—A calculated amount of calcium hydroxide was added to a 20% solution of arabic acid. *p*H of solution was 7.

Magnesium Arabate.—Magnesium carbonate (0.4 Gm.) was added to 50 cc. of a 20% solution of arabic acid and the excess filtered off.

Zinc Arabate.—A calculated amount of zinc chloride needed to neutralize a 20% solution of arabic acid was added.

Ferric Arabate.—An arabic acid solution (20%) was raised to *p*H 5 with 0.5 *N* sodium hydroxide solution. A calculated amount of a 20% solution of ferric chloride was added to the solution. No gelation would occur below a *p*H of 5.

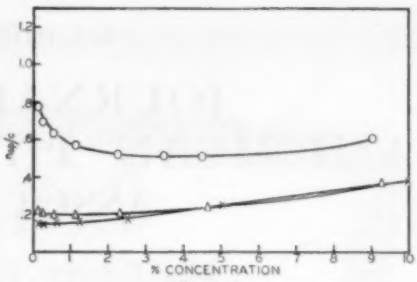


Figure 1.
○, gum arabic; Δ, gum arabic with 0.5 *M* NaCl; ×, gum arabic with 0.5 *M* $CaCl_2$.

Aluminum Arabate.—A solution (20%) of arabic acid was raised to a *p*H of 7 with 0.5 *N* sodium hydroxide. Equivalents (25×10^{-4}) of aluminum chloride in the form of a 20% solution were added. Then more 0.5 *N* sodium hydroxide was added to raise the *p*H to 7. Gelation would not occur below a *p*H of 7.

Trimethyl Benzyl Arabate.—A technical grade trimethyl benzyl hydroxide (38%) was used. A sufficient quantity was added to a 20% solution of arabic acid to raise the *p*H to 7.

Butylamine Hydroarabate.—A sufficient quantity of butylamine was added to a 20% solution of arabic acid to raise the *p*H to 7.

Ethylenediamine Hydroarabate.—A sufficient quantity of ethylenediamine was added to a 20% solution of arabic acid to raise the *p*H to 7.

Diethylenetriamine Hydroarabate.—Since there are 3 amino groups on the molecule, it would be expected that one-third of the equivalents of diethylenetriamine would be needed for neutralization as needed for butylamine, but in this case twice as much diethylenetriamine was needed than was calculated.

Triethylenetetramine Hydroarabate.—In this case one-fourth of the equivalents of triethylenetetramine would be expected to be used for neutralization as needed for butylamine, but twice this amount was actually needed.

RESULTS AND DISCUSSION

It may be noted from the curves in Figs. 2 and 3 that a considerable difference exists in the viscosity curves of sodium arabate and arabic acid without the presence of salt, but in the presence of salt the viscosity was the same. One possible explanation is that the higher viscosity of the sodium arabate was due to a higher charge on the molecule. Colloids with carboxylic acid groups lose their negative charge quite readily at a low *p*H, but in the region of neutrality ionization is practically complete, thus the charge would be high (2). The gum arabic curve (Fig. 1) without added salt was found to be between that of arabic acid and sodium arabate as might be expected from the above explanation, since gum arabic solutions have a *p*H of 5. This would be between the *p*H of 7 of the sodium arabate

and the *p*H 2.4 of arabic acid solutions; therefore, the amount of ionization should be between that of the sodium arabate and arabic acid. Of course, the fact that gum arabic is a mixture of potassium, calcium, and magnesium salts may also be a factor. The potassium salt curves (Fig. 4) were of the same magnitude and appearance as the sodium salt which was expected.

It might be expected that the curves for the divalent salts of calcium magnesium, and zinc (Figs. 5, 6, and 7), would show a higher viscosity than the monovalent salts, but this was not found to be true. In the case of the divalent salts the curves made without salt were much lower than the monovalent arabic acid salts and also did not display the rapid rise in viscosity, as shown by the monovalent salts and by arabic acid at low concentrations. Apparently, instead of an intermolecular attraction linking the carboxyl groups of two molecules, an intramolecular attraction occurred effectively linking the carboxyl groups on one molecule, thus making the molecule more compact, resulting in less hydration and lowered viscosity. The curves for the magnesium and zinc salts were of about the same magnitude and appearance as the curves for the calcium salt.

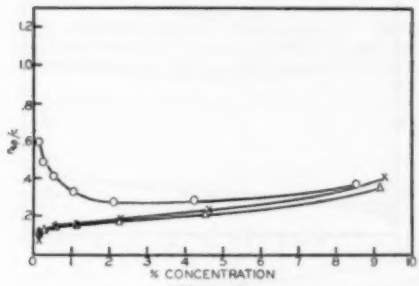


Figure 2.
○, arabic acid; Δ, arabic acid with 0.5 *M* NaCl; ×, arabic acid with 0.5 *M* $CaCl_2$.

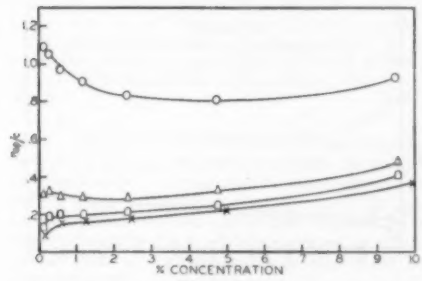


Figure 3.
○, sodium arabate; Δ, sodium arabate with 0.5 *M* NaCl; ⏢, sodium arabate with 0.5 *M* NaCl; ×, sodium arabate with 1.0 *M* NaCl.

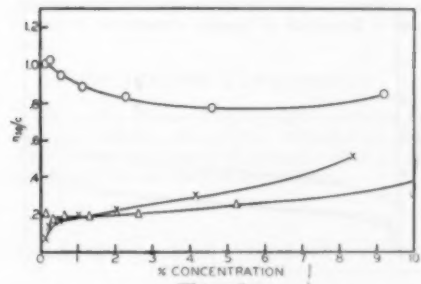


Figure 4.
○, potassium arabate; Δ potassium arabate with 0.5 *M* NaCl; ×, potassium arabate with 0.5 *M* $CaCl_2$.

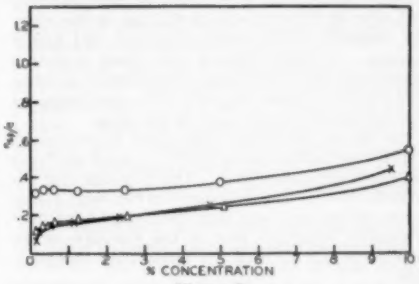


Figure 5.
○, calcium arabate; Δ, calcium arabate with 0.5 *M* NaCl; ×, calcium arabate with 0.5 *M* $CaCl_2$.

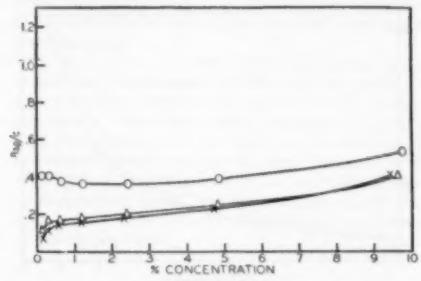


Figure 6.
○, magnesium arabate; Δ, magnesium arabate with 0.5 *M* NaCl; ×, magnesium arabate with 0.5 *M* $CaCl_2$.

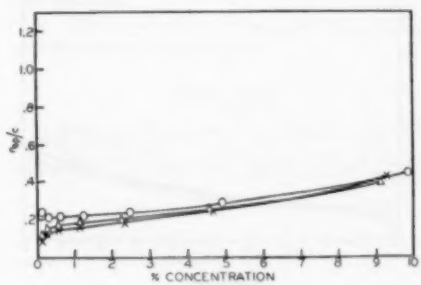


Figure 7.
○, zinc arabate; Δ, zinc arabate with 0.5 *M* NaCl; ×, zinc arabate with 0.5 *M* $CaCl_2$.

The trivalent salts might be expected to have a high viscosity. This was found to be true, as shown from the curve for the ferric salt in Fig. 8. The viscosity of the ferric arabate solution rises very rapidly in the region between 8 and 9%. The aluminum arabate viscosity curve (Fig. 9) did not rise as rapidly, but did have a high viscosity in the region of 20% concentration. The curves for both compounds decreased very rapidly at low concentrations with the curves ending up much lower than that of any of the other salts. An explanation for the results obtained is that the aluminum and ferric ion have a tendency to pull carboxyl groups toward them, and in the case of a concentrated arabic acid solution the tendency was toward an intermolecular attraction with an increase in the size of the molecule, resulting in increased hydration and higher viscosity. In dilute solution with fewer molecules present, the tendency is apparently toward an intramolecular attraction, resulting in a more compact molecule, less hydration, and low viscosity.

The curves for both butylamine hydroarabate (Fig. 10) and sodium arabate (Fig. 3) are of the same magnitude and appearance. This indicates that the butylamine salt must be ionized to the same extent as the sodium salt, and thus the charge must play an equally important part in the viscosity of the butylamine salt as with the monovalent inorganic salts.

The trimethyl benzyl arabate curve without salt (Fig. 11) has the same appearance as the monovalent salts but shows a lower viscosity. An explanation for this is that the trimethyl benzyl hydroxide used to prepare the salt has a positive charge on the molecule; therefore, it would be expected to suppress the negative charge of the arabic acid molecule and thus lower the viscosity.

The curves for ethylenediamine hydroarabate (Fig. 12), diethylenetriamine hydroarabate (Fig. 13), and triethylenetetramine hydroarabate (Fig. 14) are all similar in magnitude and appearance, and these curves are also similar to the curves obtained with the divalent inorganic salts. This would indicate that the triamine and tetramine react with

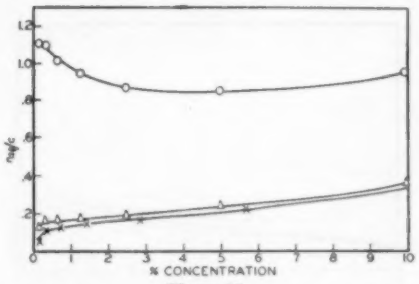


Figure 10.

○, butylamine hydroarabate; Δ, butylamine hydroarabate with 0.5 *M* NaCl; ×, butylamine hydroarabate with 0.5 *M* $CaCl_2$.

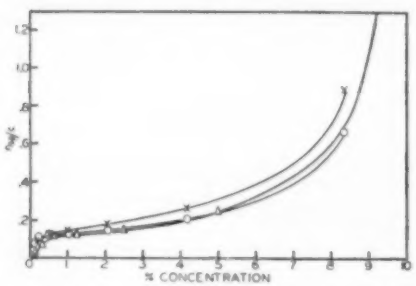


Figure 8.

○, ferric arabate; Δ, ferric arabate with 0.5 *M* NaCl; ×, ferric arabate with 0.5 *M* $CaCl_2$.

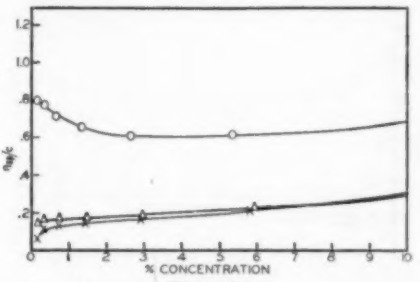


Figure 11.

○, trimethylbenzyl arabate; Δ, trimethylbenzyl arabate with 0.5 *M* NaCl; ×, trimethylbenzyl arabate with 0.5 *M* $CaCl_2$.

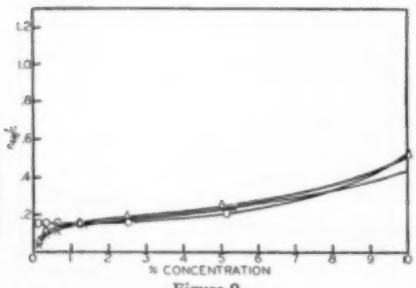


Figure 9.

○, aluminum arabate; Δ, aluminum arabate with 0.5 *M* NaCl; ×, aluminum arabate with 0.5 *M* $CaCl_2$.

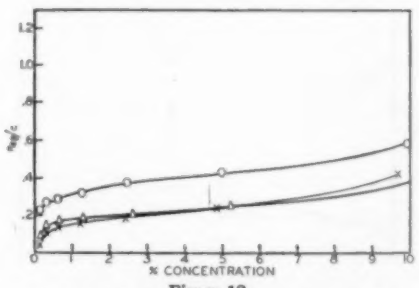


Figure 12.

○, ethylenediamine hydroarabate; Δ, ethylenediamine hydroarabate with 0.5 *M* NaCl; ×, ethylenediamine hydroarabate with 0.5 *M* $CaCl_2$.

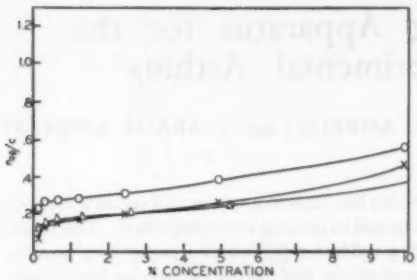


Figure 13.

○, diethylenetriamine hydroarabate; Δ, diethylenetriamine hydroarabate with 0.5 *M* NaCl; ×, diethylenetriamine hydroarabate with 0.5 *M* $CaCl_2$.

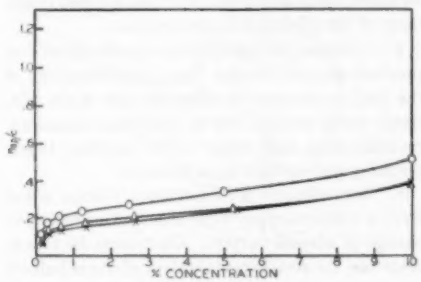


Figure 14.

○, triethylenetetramine hydroarabate; Δ, triethylenetetramine hydroarabate with 0.5 *M* NaCl; ×, triethylenetetramine hydroarabate with 0.5 *M* $CaCl_2$.

arabic acid in the same manner as ethylenediamine, and that all three compounds react like the divalent inorganic salts. It may also be observed that the curves made without the presence of salt decrease at lower concentrations rather than increase. This may probably be due to an intramolecular attraction rather than an intermolecular attraction at low concentrations.

It may be noted that sodium chloride or calcium chloride added to a solution of arabic acid, or any of its salts, lowers the viscosity curves of these solutions. Calcium chloride lowered the curves to a greater extent than sodium chloride. As might be expected, a negative charge is indicated in each case.

APPLICATION TO PHARMACY

The results discussed above may readily be applied to predicting the stability of emulsions, since the viscosity and degree of hydration play an important part in the stability of emulsions.

First of all, it was shown that the arabic acid solution had the lowest viscosity of any of the solutions considered; therefore, one might conclude that emulsions prepared with arabic acid as the emulsifying agent would cream very rapidly, for according to Stoke's law, the viscosity is a factor in the rate of creaming of an emulsion or the rate of settling of particles in a suspension.

Next, in order of increasing viscosity, were the solutions of the divalent inorganic salts and the organic salts containing two, three, and four amino groups. Emulsions made with these salts would be expected to cream at a slower rate than the arabic acid emulsions but would have a higher creaming rate than emulsions made with gum arabic, since gum arabic solutions have a higher viscosity than the solutions of the salts stated above.

Following this were the solutions of the monovalent inorganic salts and the organic salts of compounds to which one carboxyl group could be attached. These solutions had a higher viscosity than the gum arabic solutions, and, therefore, emulsions prepared with these salts would be expected to have a lower creaming rate than any of the salts already mentioned.

The solutions of the trivalent inorganic salts had the highest viscosity of all the solutions prepared at concentrations above 8–10%, but at low concentrations the viscosity was lower than the solutions of any of the other salts. From these data, the creaming rate would be expected to be very low at the higher concentrations and very high at the lower concentrations. At the higher concentrations, solutions of these salts may well serve as suspending agents.

It may also be noted from the curves shown above that the presence of salts and, in particular, divalent salts, decrease the viscosity of the arabate solutions. From these results, it may be concluded that the presence of any salts in an emulsion or suspension would produce a small decrease in the efficiency of the substance as an emulsifying agent or suspending agent, but the presence of divalent salts would cause a large decrease in the efficiency of the substances.

SUMMARY

1. Curves illustrating the effect of cations on the viscosity of arabic acid solution are shown and discussed.

2. The application of these data to emulsions and suspensions is considered.

REFERENCES

(1) Schleif, R. H., Higuchi, T., and Busse, L. W., THIS JOURNAL, 40, 98(1951).

(2) Kruyt, H. R., "Colloid Science," Vol. 2, Elsevier Publishing Co., Inc., New York, 1949, chaps. VII and VIII.

A Simple Aerosolizing Apparatus for the Production of Experimental Asthma*

By JOSEPH W. E. HARRISSON,† JULIAN L. AMBRUS,‡ and CLARA M. AMBRUS‡

An aerosol apparatus, easy to construct and applicable to the determination of the sensitivity of guinea pigs toward histamine, acetylcholine, or antigens, is described. The method described is useful to test antihistaminic, parasympatholytic, antianaphylactic, or antispasmodic drugs.

KALLOS and Pagel (1) were the first to describe a method whereby experimental asthma could be produced in guinea pigs by the inhalation of nebulized antigen or histamine. The method has been used by many investigators in numerous modifications to evaluate antianaphylactic, antihistaminic, and antispasmodic drugs (2–7). It may also be used with nebulized acetylcholine and other bronchoconstrictor substances. Some of the advantages presented by this procedure are:

a. An *in vivo* test in which the guinea pigs are not sacrificed. In fact, after a short recovery period (varying with the drug and the duration of exposure), sensitivity toward the same substance returns to normal. In this way the same guinea pig may be used as a control and as an experimental animal, which results in greater accuracy and in a smaller number of guinea pigs needed to obtain significant results. According to our experiments, the blood-antibody titer of sensitized guinea pigs does not decrease after exposure to most nebulized antigen solutions, and even several subsequent exposures do not result in significant desensitization. This is the only practical way to produce, within a short period, *in vivo* anaphylactic phenomena in the same guinea pig without change of sensitivity of the animal, which is probably due to the fact that the amount of antigen actually absorbed by the guinea pig exposed to such aerosols is minute.

b. By exposing animals at regular intervals after administration of a protective drug, the duration of action of this drug can easily be established.

c. By previous testing, guinea pigs of similar sensitivity may be selected for each experiment, resulting in greater accuracy. According to our experience, histamine and acetylcholine sensitivity of guinea pigs, 300–400 Gm. body weight, varies but little in a two-month period if they are exposed to aerosols every third day. This makes it possible for laboratories engaged in assaying products by this method, to have on hand guinea pigs of similar and of individually known sensitivity.

The apparatus used by other investigators and by ourselves in previous studies usually had one or more of the following disadvantages:

a. Commercial epinephrine nebulizers used to produce aerosols require large concentrations of the drug to provoke the effect because of the relatively small output. From high concentrations, crystallization may occur in the capillary tubes, resulting in decreased aerosol output.

b. According to our experience, change in the level of solution in any type of nebulizer results in change of aerosol output. Care must be taken, therefore, for steady replacement of the nebulized solution.

c. The closed system often used for such experiments results in a constantly increasing concentration of the nebulized substance in the exposure chamber, which makes it difficult to standardize the exposure.

d. Direct introduction of aerosols into the exposure chamber by means of the spray often produces droplets of various and inconstant size. Thus, the position of the animal in the exposure chamber may influence the amount of drug absorbed.

Lacking a more convenient and more readily controlled method, a simplified aerosol device was constructed out of the materials[1] usually available in the laboratory.

APPARATUS

Aerosol Equipment.—The aerosol equipment consists of two principal parts, i. e., the nebulizing equipment (Fig. 1, *A*) and the exposure chamber (Fig. 1, *B*). A 250-ml. Florence flask is fitted in the base with a nebulizer and liquid-leveling device. The nebulizer has 2-mm. supply vents, three of which are arranged around the base. The outer tip is approximately 3 mm. in diameter and the inner tip 1 mm. Directly opposite this is the aerosol-conducting tube, the entrance to which is protected by a baffle. This baffle is of larger di-

* Received February 7, 1951, from the LaWall Memorial Laboratory of Pharmacology and Biochemistry, Philadelphia College of Pharmacy and Science, Philadelphia, Pa.

† Director of the Laboratory.

‡ Associates.

[1] Acknowledgment is given to S. Roggenburg, Jr., for glass blowing, and to W. S. Merkins for other construction at our request.

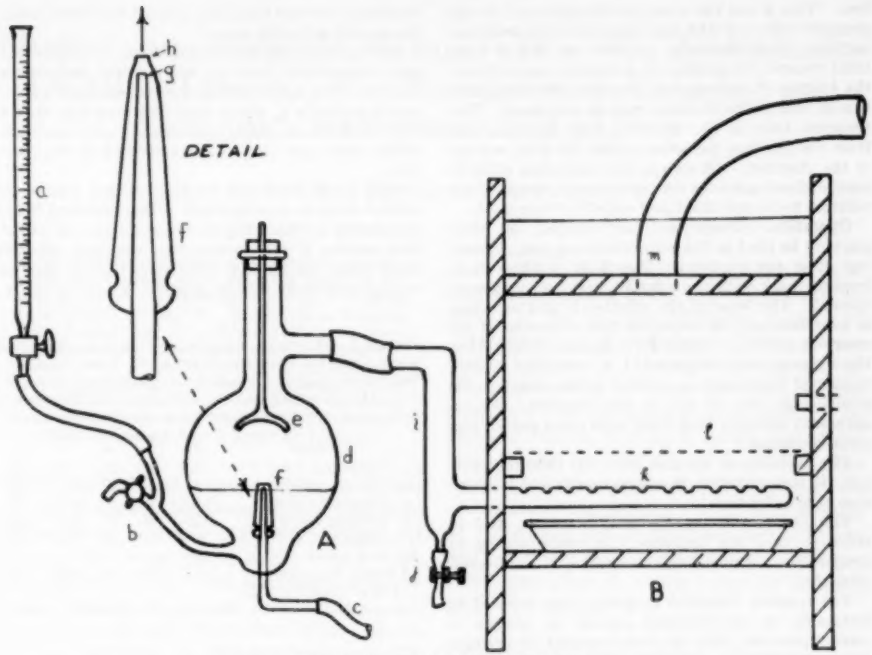


Fig. 1.—Aerosol flask and chamber.

A.—Aerosol equipment: (*a*) leveling or supply vessel; (*b*) leveling stopcock; (*c*) gas supply at which point a mercury manometer is introduced into the line; (*d*) nebulizing flask; (*e*) baffle or deflector; (*f*) nebulizer; (*g*) gas supply tip, diameter about 1 mm.; (*h*) nebulizer tip, diameter about 3 mm.; (*i*) manifold; (*j*) drainage point.

B.—Chamber: (*k*) continuation of manifold; (*l*) screen for animals; (*m*) vent to outside.

ameter than the stack. It may be conveniently constructed of circular pieces of sheet Tygon, fastened to the glass suspending rod by forcing over a beaded end. The stream of nebulized fluid first strikes this deflector, which results in the large droplets returning to the chamber and the aerosol escaping between the opening of the baffle and the flask, which opening is about 2 cm. in height. The aerosol is then conducted through the manifold in the exposure chamber. An intervening drainage point is provided for removing condensate, if necessary.

To refill the nebulizing flask, a graduated supply vessel is provided. For most operations a burette serves adequately. By this means the volume of liquid nebulized may be ascertained in any given period, and the stopcocks may be adjusted to supply material at approximately the rate of usage which will result in an approximately constant level of material in the nebulizing flask.

The supply and leveling arm is so adjusted in relation to the nebulizer that the liquid level in the nebulizer will be approximately 3–5 mm. below the top edge of the outer tip.

Exposure Chamber.—This has a chamber capacity of approximately 15 L. It is conveniently constructed of wooden ends with glass or plastic panels in face and rear, which slide in channels rabbited into the ends. The aerosol manifold is of glass approximately 1 cm. in diameter with 2-mm. vents, spaced about 1 cm. apart. A 2-in. flexible metallic tube, which leads to the outside, acts as a vent. The animals are held directly above the manifold on a screen.

The chamber may be flushed by opening the slides, installing a pump in the vent tube, or, when essential, by use of a vacuum line.

Standardization.—Each apparatus must be standardized for the amount of histamine (or other material) nebulized per minute. This may be done by introducing a definite amount and concentration of histamine solution into the nebulizer and by determining the residual amount of histamine in the solution after a definite time period of nebulization with a definite gas pressure by determining the total solids. The amount of gas passing through the nebulizer must be held within a restricted rate. Commercial reducing valves with auxillary diaphragm valves to provide a constant rate of gas flow in liters per minute will be found useful. However, the equipment should also have in the gas line a mercury manometer which must be constantly observed during operation.

Using bottled gas and adjusting a commercial gauge to deliver 6 L. per minute, results in approximately 110 mm. of mercury pressure in the nebulizer

line. This is not the same for all apparatus, as the pressure will vary with the diameter of the nebulizer outlets. From the data, gas flow per unit of time, total volume (or weight) of nebulized material and the volume of the exposure chamber, the concentration of drug in the chamber may be calculated. The turnover time in the chamber may be calculated from the gas flow per minute and the total volume of the chamber. Of course, the apparatus must be standardized specially for each concentration of the solution to be nebulized and each flow rate used.

Operation.—Compressed air, oxygen, or other gas may be used as the aerosol-forming gas, depending upon the problem. The flask is filled to its leveling mark with the solution, and the gas supply opened. The level of the solution in the nebulizer is kept constant by adjusting the stopcocks of the reservoir and the constant-level draining tube. After the turnover time is passed, i. e., constant concentration of the aerosol is reached in the chamber, the guinea pigs may be put in the chamber. An experienced operator may work with three guinea pigs simultaneously.

The variables in the test are: (*a*) time of exposure, (*b*) concentration of solution used, and (*c*) pressure of nebulization.

The pressure is usually held constant, and in order to vary the exposure, the concentration of drug in the nebulized liquid is changed as deemed advisable.

The reaction observed in guinea pigs exposed to histamine or acetylcholine aerosol or antigen is rated upon the basis of time required to produce arbitrary responses as follows:

(*a*)	Hypernea	±
(*b*)	First forced cough	+
(*c*)	Dyspnea	++
(*d*)	Reeling	+++
(*e*)	Collapse	++++

Symptoms normally occur and progress in the above order (*a* to *e*), with an occasional guinea pig reversing the *b* and *c* responses. Reproducibility is best obtained by averaging the time required to produce the *b* and *c* (+ and ++) reactions. The mathematical mean of these two reaction times, in seconds, is termed the "aerosol reaction time," and this is used in reporting data. Exposures of this magnitude to histamine do not result in fatality, a situation which develops in a certain number of guinea pigs if exposure is carried to the *e* (++++) stage, which is often used by investigators for evaluating purposes. When no reaction occurs within ten minutes the response is indicated as negative and recorded as zero (0). In such cases higher concentrations of the solution must be used after about twenty minutes.

Guinea pigs are removed from the chamber immediately following development of the *d* (+++) reaction. Under such circumstances, some proceed into the *e* (++++) stage after removal from the chamber, but this lasts only a short time and fatalities do not generally occur.

The typical "aerosol reaction time" of untreated pigs varies from thirty to one hundred and eighty seconds. The entire course of some reactions is illustrated in Table I, where data on guinea pigs are set forth to illustrate typical individual variations of reaction times and characteristic courses of the reaction.

Due to the variation between guinea pigs, each animal must be standardized. This standard holds reasonably constant for the same animal for about two months if the guinea pigs are not exposed more often than every third day. Guinea pigs of similar sensitivity may be grouped in any one study.

TABLE I.—TYPICAL REACTION TIMES IN SECONDS WITH GUINEA PIGS OF DIFFERENT TIME CHARACTERISTICS [0.2% Histamine Diphosphate Aerosol, 6 L./Min. Compressed Air (110 Mm. Hg Pressure)]

Reaction	Guinea Pig No. 1	2	3	4	5
(*a*) Hypernea (±)	35	15	25	70	25
(*b*) Forced cough (+)	120	120	35	150	100
(*c*) Dyspnea (++)	150	80	45	180	60
(*d*) Reeling (+++)	240	150	75	360	125
(*e*) Collapse (++++)		180	85	390	140
Av. of *b* and *c* "Aerosol Reaction Time" (ART 0.2%)	135	100	40	165	80

SUMMARY

1. An easily constructed aerosol apparatus is described.

2. This apparatus may be employed to determine the sensitivity of guinea pigs toward histamine, acetylcholine or antigens to which the animals are sensitized.

3. An arbitrary means is suggested for evaluating the response of guinea pigs to such aerosols and defined as the "aerosol reaction time."

4. This method is useful to test antihistaminic, parasympatholytic, antianaphylactic, or antispasmodic drugs.

REFERENCES

(1) Kallos, P., and Pagel, W., *Acta. Med. Scand.*, **91**, 202 (1937).
(2) Issekutz, B. V., and Gennerisch, P., *Arch. expil. Path. Pharmakol.*, **202**, 201(1943).
(3) Schaumann, O., *ibid.*, **196**, 109(1940).
(4) Neely, F. L., *J. Lab. Clin. Med.*, **27**, 319(1941).
(5) Bovet, M. D., and Waltheret, F., *Ann. pharm. franç.*, Supplement 2, No. 4, 1944.
(6) Loew, E. R., Kaiser, M. E., and Moore, V., *J. Pharmacol. Expil. Therap.*, **83**, 120(1945).
(7) Winter, C., *ibid.*, **90**, 224(1947).

Studies on *Mahonia* Genus. IV. *M. Leschenaultii* (Wall.) Takeda, *M. Manipurensis* Takeda, and *M. Sikkimensis* Takeda*

By R. CHATTERJEE and M. P. GUHA†

A chemical examination of some of the species of *Mahonia* growing in different parts of India, e. g., *M. Leschenaultii* (Wall.) Takeda from South India, *M. manipurensis* Takeda from Assam, and *M. sikkimensis* Takeda from Sikkim, has yielded different alkaloids in different quantities. Comparative morphological, histological, and chemical studies have revealed that it is well to consider *M. manipurensis* Takeda as a variety of *M. borealis* Takeda rather than a separate species.

THE GENUS *Mahonia* comprises about 62 species which are distributed mainly over Asia and America. As a result of the study of the specimens in the herbariums of Indian Botanic Garden, Sibpur, Calcutta, and Botanical Survey of India, Calcutta, it is found that 23 species are from China and only 11 are from India, and the rest are scattered in different countries. In China, most of the species are found in Yunnan, and so it seems that Yunnan is the epicenter of *Mahonia* from where it is distributed to Assam, where the largest number of Indian species is obtained, and gradually it diffuses to Sikkim and Nepal.

In Table I are given the geographical distributions of Indian species of *Mahonia*. The present chemical examination has been undertaken with material from these geographical areas.

It appears that the number of species increases in the Eastern Himalaya. In Assam alone, five of the species are obtained. Only one species, *M. Griffithii* Takeda, occurs in Bhutan. One species, *M. Leschenaultii* (Wall.) Takeda, grows in South India. Formerly this was considered to be a variety of *M. nepalensis* (1). Its distribution is confined mostly to Nilgiri hills, where it grows in abundance, and to Annamalai hills, Tinnevelly hills, and Ootacamund.

The present work deals mainly with the isolation and identification of the alkaloids of the three species of *Mahonia* growing in different parts of India, Assam, Sikkim, and South India. Our objectives have been: (*a*) to find out if the results of the previous alkaloidal findings on *Mahonia*, e. g., *M. nepalensis* D. C., growing largely in Nepal (2), *M. Griffithii* Takeda occurring in Bhutan (3), *M. acanthifolia* Don, the common species of the Eastern Himalayas (4), *M. Simonsii* Takeda, growing in Assam, and *M. borealis* Takeda, growing largely in Dehra Dun, Northwestern India (5), can be correlated with the results of the chemistry of *M. manipurensis*, *M. sikkimensis*, and *M. Leschenaultii;* and (*b*) to find out if the habitat influences the formation of different alkaloids, either in quality or quantity.

TABLE I.—THE GEOGRAPHICAL DISTRIBUTION OF INDIAN SPECIES OF *Mahonia*

1. Eastern India—Assam	*M. acanthifolia, M. calamicaulis, M. manipurensis, M. pycnophylla, M. Roxburghii, M. Simonsii*
2. Northern India:	
(*a*) Nepal and Darjeeling	*M. acanthifolia, N. nepalensis*
(*b*) Sikkim	*M. acanthifolia, M. sikkimensis*
(*c*) Bhutan	*M. Griffithii*
3. Southern India	*M. Leschenaultii*
4. Northwestern India	*M. borealis*

Takeda has differentiated *M. Leschenaultii* from *M. nepalensis* in consideration of the difference in some parts of their flowers (6). The chemical study has substantiated the morphological difference of these plants, for the roots of the former have furnished a higher percentage of berberine (1.02 per cent), whereas the percentage yield of umbellatine in the latter case does not exceed 0.5 per cent. Besides berberine, four other alkaloids, oxyacanthine (in traces), neprotine (0.011 per cent), palmatine (0.075 per cent), and jatrorrhizine (0.031 per cent) have been isolated from *M. Leschenaultii*. It is interesting to note that although the amount of berberine has been appreciably increased, the quantities of other alkaloidal constituents in *M. Leschenaultii* have not increased in the same proportion.

* Received October 12, 1950, from the Department of Chemistry, Presidency College, Calcutta, India.
†The authors are indebted to Dr. P. C. Rakshit, Head of the Department of Chemistry, Presidency College, Calcutta, for providing laboratory facilities. They gratefully acknowledge the supply of the roots of *M. Leschenaultii* (Wall.) Takeda collected by Mr. V. Narayanaswami of the Botanical Survey of India from Ootacamund, the stem bark of *M. sikkimensis* Takeda by Mr. B. Pradhan of Sikkim, and the roots of *M. manipurensis* Takeda by Dr. S. K. Mukerjee during his Botanical Expedition to Manipur State in 1947–1948.

The stem bark of *M. sikkimensis* has furnished only three alkaloids, i. e., oxyacanthine (in traces) berberine (0.455 per cent), and neprotine (0.0009 per cent). This species is thus different from other morphologically allied species, e. g., *M. acanthifolia*, *M. borealis*, and *M. manipurensis*, since the authors have already reported the presence of five alkaloids in *M. acanthifolia* (4), whereas an examination of the roots of *M. borealis* (5) in this laboratory has indicated the presence of the same five alkaloids in different proportions.

Four alkaloids were obtained from the roots of the species *M. manipurensis*. The morphological and histological studies of the two species, *M. borealis* Takeda and *M. manipurensis* Takeda, have shown very little difference (5). The similarity of these two species appears to be more striking, for they synthesize the alkaloids in nearly the same relative proportion, i. e., in the roots of both the species the per cent of berberine is slightly less than that of neprotine, and, therefore, it seems well to consider *M. manipurensis* as a variety of *M. borealis*, rather than a separate species.

A summary of the alkaloids of the different species now under review is given in Table II, and the relative yields of the major alkaloids, berberine and neprotine, have been compared in Table III.

EXPERIMENTAL

Finely powdered root or stem bark (1 Kg.) was extracted exhaustively with either cold or hot ethanol (95%). The alcohol of the extract was removed under reduced pressure and the residue treated with iced water (500 cc.) and filtered three times to get rid of the resinous matter. The clear dark brown liquid was cooled and made alkaline with ammonia (25%). A sticky amorphous solid separated and the mixture was extracted five times with ether. In some cases, the ethereal layer separated with difficulty, due to the formation of an emulsion with the sticky matter.

Treatment of the Ethereal Layer

The yellow-colored ether layer was washed twice with water (10 cc. each time) and the ether then removed. A sticky mass was obtained (1.7 Gm.). The sticky mass was Soxhleted with dry ether (50 cc.), whereupon most of it went into solution. The solution was filtered and left for a day or two in a refrigerator. Colorless crystals, which were sticky with resinous matter, separated. The crude crystals were treated with a small quantity of dilute hydrochloric acid (5%), filtered from insoluble matter, and allowed to stand in the refrigerator for a long time. Crystals of a hydrochloride then appeared.

The Crystals: Identification of Oxyacanthine.—The pale yellow crude hydrochloride was dissolved in warm water and treated carefully with ammonia, so that an amorphous oxyacanthine base was obtained. The base was taken up in ether, and the ethereal solution, on long standing in refrigerator, yielded colorless crystals (in traces) which, on recrystallization from methanol, melted at 216° in vacuum and did not depress the melting point of an authentic sample. On adding a drop of ferric chloride solution, followed by potassium ferricyanide, to the base, a green coloration was obtained which rapidly changed into blue. This is the characteristic color reaction of oxyacanthine.

All three species, *M. Leschenaultii*, *M. sikkimensis*, and *M. manipurensis*, yielded this base in traces.

The Mother Liquor.—The mother liquor was cooled and treated with sodium nitrate solution. No crystals of berberine nitrate were obtained as had been obtained from *M. Griffithii* (3).

TABLE II.—A COMPARATIVE STUDY OF THE ALKALOIDS OF THE INDIAN SPECIES OF *Mahonia*

Species	Locality	—Yield of Alkaloids in Gm./Kg. of Plant Material— Free Bases from Ether Layer	Alkaloids from Hydrochloride Fraction	Reduced Bases from Iodide Fraction
M. nepalensis	Darjeeling	...	4.8[a]	...
			0.2[b]	...
M. acanthifolia	Darjeeling	Traces[g]	0.075[b]	0.71[d]
			5.3[c]	1.4[f]
M. Griffithii	Bhutan	Traces[g]	Traces[b]	Traces[d]
		Traces[h]	3.0[c]	...
M. sikkimensis	Sikkim	Traces[g]	0.01[b]	...
			4.8[c]	...
M. Leschenaultii	Nilgiri Hills	Traces[g]	0.12[b]	0.76[d]
			10.8[c]	Traces[e]
				0.32[f]
M. Simonsii	Manipur (Assam)	Traces[g]	2.91[b]	0.23[d]
			0.22[c]	0.194[f]
M. manipurensis	Manipur (Assam)	Traces[g]	Traces[b]	Traces[f]
			Traces[c]	...
M. borealis	Dehra Dun	Traces[g]	3.4[b]	0.3[d]
			1.7[c]	0.364[f]

[a] Umbellatine hydrochloride. [b] Neprotine hydrochloride. [c] Berberinium chloride. [d] Tetrahydropalmatine.
[e] Tetrahydroberberine. [f] Tetrahydrojatrorrhizine. [g] Oxyacanthine. [h] Berbamine.

TABLE III.—AMOUNTS OF BERBERINE IN RELATION TO NEPROTINE AS OBTAINED FROM INDIAN *Mahonia* SPECIES

Species	Locality	Yield of Berberinium Chloride in Gm./Kg. of Plant Material	Yield of Neprotine Hydrochloride in Gm./Kg. of Plant Material	Approx. Ratio of Berberine to Neprotine
M. acanthifolia	Darjeeling	5.3	0.075	70:1
M. Griffithii	Bhutan	3.0	Traces	3:0
M. sikkimensis	Sikkim	4.8	0.01	480:1
M. Leschenaultii	Nilgiri Hills	11.0	0.12	100:1
M. Simonsii	Manipur (Assam)	0.22	2.91	1:13
M. manipurensis	Manipur (Assam)	Traces	Traces	Berb. < neprotine
M. borealis	Dehra Dun	1.7	3.4	1:2

Treatment of the Aqueous Layer

The clear brown aqueous solution (600 cc.) was treated with hydrochloric acid to give a 1% solution, and was allowed to stand overnight in the refrigerator. A mixture of yellow and red hydrochlorides gradually crystallized out, and this was filtered off.

Treatment of the Crystals: Separation of Berberinium Chloride from Neprotine Hydrochloride in the Case of *M. Leschenaultii*.—The alcoholic solution of the crude mixture of the hydrochlorides (11.4 Gm.) was allowed to crystallize slowly after treatment with activated charcoal to separate yellow crystals of berberinium chloride (10.8 Gm.) and those of red neprotine hydrochloride (0.12 Gm.).

Identification of the Yellow Crystals as Berberinium Chloride.—The yellow crystals were further purified by repeated crystallizations from dilute ethanol and did not show any definite melting point, but charred on heating. The product (0.3 Gm.) was then dissolved in warm water containing a little hydrochloric acid. It was treated with platinic chloride (0.15 Gm.) to precipitate the yellow chloroplatinate, and this was washed well with water until free from hydrochloric acid. Found: Pt, 17.7 $(C_{19}H_{17}O_4N)_2H_2PtCl_6$ requires Pt, 18.02%.

This indicated that the yellow hydrochloride might be berberinium chloride. It was further confirmed by the preparation of the following derivatives.

Anhydroberberineacetone.—The pure yellow hydrochloride (0.1 Gm.) was dissolved in water (10 cc.) and was mixed with 10% aqueous sodium hydroxide solution (2 cc.), warmed to 50° and mixed with acetone (5 cc.), and then allowed to cool to the room temperature, when lemon yellow crystals of the acetone compound (0.065 Gm.) gradually separated. The crystals melted at 167–169°, and did not depress the melting point of an authentic sample of anhydroberberineacetone.

Tetrahydroanhydroberberine.—The yellow hydrochloride (4 Gm.) was reduced by zinc dust and glacial acetic acid until the solution was faintly yellow, cooled, and treated with ammonia until the precipitated zinc hydroxide redissolved and an amorphous residue of the reduced base was left behind. The mixture was then repeatedly extracted with ether and, on removing ether, crystals of a colorless compound (2.9 Gm.) were obtained. The colorless product on recrystallization from methanol melted at 172–173° and did not depress the melting point of an authentic specimen of tetrahydroanhydroberberine, m. p. 173°.

Identification of the Red Crystals as Neprotine Hydrochloride.—The crude red hydrochloride was purified by repeated crystallization from dilute ethanol and was obtained as beautiful red elongated prisms on slow crystallization. The crystals charred without showing any definite melting point.

An ethanolic solution of picric acid (1 mole) was added to a warm ethanolic solution of the red hydrochloride (1 mole). On cooling, shining orange-colored crystalline needles of the picrate were obtained. The picrate melted at 208–210° decompd., and did not depress the melting point of an authentic sample of neprotine picrate similarly prepared from *M. nepalensis* (2, 3).

Separation of Berberinium Chloride from Neprotine Hydrochloride in the Case of M. sikkimensis.—This species also behaved in the same manner and furnished 5.2 Gm. of the crude mixture of hydrochlorides at this stage, and from the mixture the yellow and the red crystals could be easily separated, as outlined before. The yellow crystals (4.8 Gm.) were identified with berberinium chloride by preparing the derivatives—anhydroberberineacetone, m. p. 167–169° decompd., tetrahydroanhydroberberine, m. p. 172–173°, and the chloroplatinate salt, which on analysis gave Pt, 17.94%. The red product (0.01 Gm.) was identified with neprotine hydrochloride by preparing the orange yellow picrate, m. p. 208–210° decompd., which did not depress the melting point of an authentic sample.

Treatment of the Aqueous Layer in the Case of M manipurensis.—This species, however, behaved in a different manner and did not furnish any solid hydrochloride, even on standing in the refrigerator for a number of days. The species is obviously very poor in alkaloid content and in the first experiment the mother liquor was further treated according to the general procedure for the precipitation of the iodides. The absence of neprotine from it appeared doubtful, for it is found that neprotine is a commonly occurring alkaloid in all the Indian species so far studied (Table II). This necessitated further confirmation, and consequently the method was modified with a view to identify neprotine, even if present in traces.

In this case the aqueous layer was directly treated with a saturated solution of picric acid so as to precipitate a mixture of orange yellow picrates. This was dissolved in dilute ethanol and on slow crystallization furnished a few beautiful orange needles, m. p. 208–210° decompd., which did not depress the melting point of an authentic specimen of neprotine picrate. Another crop of yellow silky

needles was subsequently obtained from the mother liquor. These were, in all probability, crystals of berberine picrate, but further study was not possible, due to paucity of material.

Treatment of the Filtrate Obtained from the Aqueous Layer: *In the Case of M. Leschenaultii.*—The filtrate (600 cc.), after the separation of berberine and neprotine hydrochlorides, was cooled in ice and treated with sodium acetate solution (10 Gm.), followed by a solution of potassium iodide added in excess, and a brown iodide (5.8 Gm.) was precipitated. It was then filtered and the filtrate rejected.

In the Case of M. sikkimensis.—This species, however, produced an extremely sticky product, which could not be successfully worked up for the identification of any other alkaloid.

In the Case of M. manipurensis.—The iodide of this species also had a similar tendency to become sticky, but on careful treatment with a little ethanol the sticky matter slowly dissolved and, on filtration, the iodide (2.8 Gm.) was obtained in a comparatively pure condition.

Action of Zinc and Glacial Acetic Acid upon the Brown Iodide, Obtained from *M. Leschenaultii*

The precipitated brown iodide (5.8 Gm.) was suspended in water (200 cc.) and was digested with freshly precipitated silver chloride (10 Gm.) by heating on a sand bath for about sixteen hours, the mixture being agitated by means of a mechanical stirrer. The chlorides of the bases formed by double decomposition went into solution, being more soluble than the iodides. The mixture was finally filtered hot, and the dark brown solution was immediately reduced by zinc dust and a mixture of glacial acetic acid (30 cc.) and concentrated hydrochloric acid (25 cc.) by warming on a water bath until the color of the solution became very pale yellow. The reduced solution was filtered from excess of unreacted zinc, cooled in ice, and was treated with ammonia until the precipitated zinc hydroxide dissolved again, leaving behind an amorphous precipitate of the reduced bases. The mixture was further cooled in ice and then filtered. The filtrate was extracted several times with ether. The pale yellow base was dissolved, as far as possible, by vigorous shaking with ether and both the ether layers were mixed together, washed well with water, followed by washing with 5% sodium hydroxide (30 cc.) in small installments. The ethereal layer was then washed with ether until free from alkali.

The Ether Solution: Separation of Tetrahydroberberine from Tetrahydropalmatine.—The residue obtained on removing ether was crystallized from methanol to yield colorless crystals, m. p. 130–135°. The product was then fractionally crystallized until two fractions, exhibiting sharp melting points, could be separated. The first fraction (0.76 Gm.), m. p. 142–143°, did not depress the melting point of an authentic sample of tetrahydropalmatine melting at 144°. The second fraction, obtained only in traces, melted at 172°, and did not depress the melting point of an authentic specimen of tetrahydroanhydroberberine.

The Alkaline Solution: Identification of Tetrahydrojatrorrhizine.—The alkaline solution (30 cc.) was dark brown in color, indicating the presence of phenolic base. The solution was cooled in ice and acidified carefully with concentrated hydrochloric acid added dropwise, and the hydrochloride was precipitated as a pale yellow solid. The mixture was subsequently treated with ammonia and the free base taken up in ether. The colorless residue left on removing ether was crystallized from methanol and crystals thus obtained (0.32 Gm.) melted at 205–206° decompd., no depression in melting point being observed when mixed with a sample of tetrahydrojatrorrhizine similarly prepared from *M. acanthifolia* (4).

Action of Zinc and Glacial Acetic Acid upon the Brown Iodide, Obtained from *M. manipurensis*

The iodide fraction (2.8 Gm.) was worked up in the same manner and ultimately separated into two fractions—nonphenolic and phenolic. Only tetrahydroanhydroberberine could be isolated in traces from the nonphenolic part, whereas the phenolic part yielded traces of tetrahydrojatrorrhizine.

SUMMARY

1. Alkaloids have been isolated from three species of *Mahonia* growing in different parts of India, i. e., *M. Leschenaultii* (Wall.) Takeda from South India, *M. manipurensis* Takeda from Assam, and *M. sikkimensis* Takeda from Sikkim.

2. It has been found that the roots of *M. Leschenaultii* contain oxyacanthine (in traces), berberine (1.02 per cent), neprotine (0.011 per cent), palmatine (0.075 per cent), and jatrorrhizine (0.031 per cent). The roots of *M. manipurensis* contain very small quantities of oxyacanthine, berberine, neprotine, and jatrorrhizine. The stem bark of *M. sikkimensis* has furnished oxyacanthine (in traces), berberine (0.46 per cent), and neprotine (0.001 per cent).

3. It has been suggested that *M. manipurensis* Takeda should preferably be called a variety of *M. borealis* Takeda, rather than a separate species, as both of them show great chemical, morphological, and histological similarities.

REFERENCES

(1) Hooker, J. D., "Flora of British India," Vol. I, L. Reeve & Co., London, 1875, p. 109.
(2) Chatterjee, R., THIS JOURNAL, 33, 210(1944).
(3) Chatterjee, R., and Guha, M. P., *ibid.*, 39, 181(1950).
(4) Chatterjee, R., and Guha, M. P., *ibid.*, 39, 577(1950).
(5) Chatterjee, R., Guha, M. P., and Sen, S. K., *ibid.*, 40, 36(1951).
(6) Takeda, H., *Notes Bot. Gard. Edin.*, 6, 209(1911–1917).

Studies on *Mahonia* Genus. V. Structure of Neprotine*

By R. CHATTERJEE and M. P. GUHA†

The alkaloid neprotine is a secondary base and contains two methoxyl, two phenolic, and two alcoholic groups. A structural formula (VI) is suggested for it.

THE BASE neprotine, $C_{18}H_{23}O_6N$, was first discovered in *Mahonia nepalensis* D. C. (1). It was subsequently found to occur in several species of *Mahonia*, growing in different parts of India, and the amount of the base appeared to vary with the species. Table I shows the variation of the neprotine content of the different species of *Mahonia* examined so far.

TABLE I.—THE PER CENT OF NEPROTINE IN DIFFERENT SPECIES OF *Mahonia*

Species	Parts Examined	Neprotine, %	References
M. nepalensis	Root	0.020	(1)
M. Griffithii	Bark	0.001	(2)
M. acanthifolia	Root	0.007	(3)
M. borealis	Root	0.311	(4)
M. Simonsii	Root	0.270	(4)
M. Leschenaultii	Root	0.011	(5)
M. sikkimensis	Bark	0.001	(5)
M. manipurensis	Root	Traces	(5)

The Nitrogen Atom.—The presence of an imino group in a molecule of neprotine has been proved by the formation of a phenyl thiocarbamide derivative, as used in the case of laurotetanine (6), as well as actinodaphnine (7).

The Oxygen Atoms.—Neprotine does not yield any derivative characteristic of the compounds containing carboxyl groups, nor does it respond to the reaction of methylenedioxy group, according to Clowes and Tollens (8). The presence of two methoxyl groups in a molecule of the base has, however, been ascertained by Zeisel's method (9).

The alkaloid gives a faint greenish yellow color with ferric chloride and dissolves in caustic alkali, producing a deep red color. On treatment of the free base in alkaline solution with dimethyl sulfate, a yellow product is obtained which is found to contain four methoxyl groups; this indicates the presence of two phenolic hydroxyl groups in a molecule of neprotine.

When treated with methyl magnesium iodide in anisole (10), neprotine yields methane equivalent to five hydrogen atoms in one molecule of the base, together with one extra atom of hydrogen from its molecule of water of crystallization, whereas methylated neprotine, on similar treatment, yields methane corresponding to only two hydrogen atoms. The results can well be accounted for, since the two free phenolic groups and the imino group, which can yield methane equivalent to three hydrogen atoms, are methylated by dimethyl sulfate. It appears, therefore, that in neprotine, there are two more hydroxyl groups, probably alcoholic in nature. With due consideration to the function of one nitrogen and six oxygen atoms, the formula of the base can now be extended to

$$C_{17}H_{13}\begin{cases}\text{—NH}\\ \text{—(OMe)}_2\\ \text{—(OH)}_2 \text{ phenolic}\\ \text{—(OH)}_2 \text{ alcoholic}\end{cases}$$

The Nucleus.—On heating neprotine hydrochloride with zinc dust in a current of hydrogen, isoquinoline is obtained, which shows undoubtedly that it belongs to the isoquinoline group of alkaloids. Since neprotine occurs with berberine in plants, it is assumed that the former is structurally allied to the latter, and it is found that the spectrum of berberinium chloride shows a continuous absorption beginning from 4,700 Å. to the ultraviolet limit, so also the spectrum of neprotine hydrochloride, which has the same type of continuous absorption, though the limit of absorption is shifted to the wave-length region of 4,850 Å. It appears, therefore, like berberine and other alkaloids with which it is associated in the *Mahonia* plants, neprotine has essentially a tetrahydroisoquinoline nucleus in the molecule.

The isolation of orthohemipinic acid (I) on oxidation of neprotine with potassium permanganate and alkali, and of opianic acid (II) on oxidation with manganese dioxide and sulfuric acid, indicate that both the products must have originated from a substituent benzyl group similar to those noticed in the molecules of narcotine (11) and umbellatine (12). Both the products of oxidation show that the two methoxyl groups in neprotine are present in the same benzene nucleus

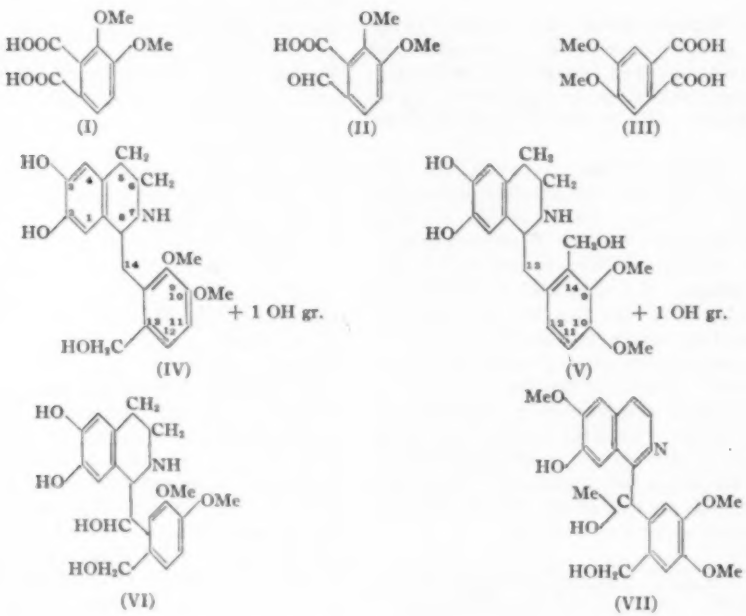
* Received October 12, 1950, from the Department of Chemistry, Presidency College, Calcutta, India.
† The authors are indebted to Dr. P. C. Rakshit, Head of the Department of Chemistry, for laboratory facilities.

and in adjacent positions. It may also be added that the aldehyde group in opianic acid has developed from the primary alcoholic group present in the same benzene nucleus with the two methoxyl groups, and the production of the carboxyl group indicates the fission of the link of this benzene nucleus to the tetrahydroisoquinoline ring.

The nitrogen fragment, however, could not be isolated from the products of oxidation of neprotine. This is attributed to the instability of the benzene ring of the tetrahydroisoquinoline part of the molecule, due to the presence of phenolic hydroxyl groups. That such a ring system became susceptible to fission by oxidizing agents had been observed by previous workers; e. g., Spaeth and Dobrowsky (13) apprehended that the benzene nucleus which contained the free phenolic hydroxyl group in corybulbine would be destroyed by oxidizing agents unless protected by alkylation. In alizarin, the benzene ring which contained hydroxyl groups was completely destroyed on oxidation with manganese dioxide and sulfuric acid. In the alkaloid umbellatine (12), the nitrogenous portion of the molecule appeared to be destroyed by oxidizing agents, as it bore phenolic hydroxyl groups.

In a subsequent oxidation with potassium permanganate and alkali, therefore, the phenolic hydroxyl groups in neprotine have been protected by methylation, and consequently metahemipinic acid (III), in addition to orthohemipinic acid, is obtained. The isolation of metahemipinic acid locates the two phenolic hydroxyl groups in neprotine in positions 2 and 3 of the tetrahydroisoquinoline skeleton, since the positions of the carboxyl groups in metahemipinic acid can now be said to be 5 and 6 in relation to the 2 and 3 positions of the methoxyl groups derived from the tetrahydroisoquinoline portion of the methylated neprotine molecule.

The Linking of the Benzene Nucleus to the Isoquinoline Nucleus.—The alkaloids with which neprotine is found to be associated in nature are all found to have the benzyl group attached to the tetrahydroisoquinoline part of the molecule through the eighth carbon atom. A similar linkage may, therefore, be attributed to neprotine. The production of opianic acid indicates for neprotine either a benzyl tetrahydroisoquinoline structure with a substituent in the *ortho* position to the linkage which is oxidized to the aldehyde group, or a hydrastine-like structure with a lactonic group. Neprotine, however, has not been found to undergo hydrolytic decomposition, and such a lactonic structure for neprotine cannot explain the existence of five active hydrogen atoms in the molecule. Again, on titrating methylated neprotine with a stand-



ard solution of alkali, it is observed that there is no reaction, which indicates the absence of any free or potential carboxyl group in its molecule.

The Positions of the Alcoholic Hydroxyl Groups.—The positions 2 and 3 are occupied by the two phenolic hydroxyl groups. The aldehyde group of opianic acid is developed in position 13 of Formula IV, after fission of the linkage and consequent development of a carboxyl group at 14. The aldehyde group is almost certainly obtained by the oxidation of the primary alcoholic group in 13. The structure of neoprotine is, therefore, preferably represented by the skeletal Formula IV with a primary alcoholic group in position 13, for the location of this group in 14 of Formula V cannot explain the formation of opianic acid.

So far it has been possible to locate the positions of all the functional groups except one alcoholic group which may be placed at any one of the positions 5, 6, and 14 of Formula IV, alalthough the positions 5 and 6 appear to be less likely and can probably be eliminated, as no alkaloid with an alcoholic hydroxyl group in either of these two positions has been isolated so far in the entire group of isoquinoline alkaloids (14). On the other hand, with due consideration to hydrastine which is associated with berberine in both *Hydrastis canadensis* L. (15) and *Berberis laurina* Billb. (Thunb.) (16), and which has been found to contain a latent alcoholic hydroxyl group in the position corresponding to 14 in skeleton Fromula IV, the remaining hydroxyl group in neprotine may reasonably be placed at 14 of Formula IV so that the structural formula for neprotine may finally be represented by Formula VI. It is quite likely that the hydroxyl group in 14 will be inert to usual reagents for hydroxyl groups. Haworth and Perkin (17) had stated that all attempts to methylate and acetylate an allied base (VII) had been unsuccessful. The two hydroxyl groups in adjacent positions 2 and 3, in the isoquinoline moiety of an alkaloid, though rare, is not without precedence, for Girardet (18) has shown the presence of two adjacent phenolic hydroxyl groups in laurepukine.

The base neprotine is optically inactive; structural Formula VI suggests that it should have both *d*- and *l*- forms, and it is yet to be seen if these forms can be isolated.

EXPERIMENTAL

Action of Phenyl Isothiocyanate on Neprotine.—The free base neprotine (1 mole) was dissolved in ethanol, and phenyl isothiocyanate in ethanol was added. On standing, crystals of phenylthiocarbamide separated, which decomposed without melting above 200°. Found: N, 5.2. $C_{19}H_{22}O_6N.SCNHC_6H_5$ requires N, 5.6%.

Action of Dimethyl Sulfate on Neprotine.—Neprotine (0.36 Gm.) was dissolved in 10% potassium hydroxide solution and shaken vigorously with excess of freshly distilled dimethyl sulfate, when a yellow methylated product separated, and was crystallized from ethanol as yellow silky needles, which decomposed without melting above 200°. Found in a sample dried in a vacuum desiccator: OMe, 30.53. $C_{19}H_{14}O_3(NMe)(OMe)_4$ requires OMe, 30.76%.

Action of Methyl Magnesium Iodide on Neprotine.—Neprotine (0.0325 Gm.) in dry anisole was treated at the ordinary temperature with excess of methyl magnesium iodide to yield methane (10.01 cc. at normal temperature and pressure). $C_{17}H_{13}(NH)(OMe)_2(OH)_4$, H_2O produces 11.53 cc. of methane at normal temperature and pressure, equivalent to six atoms of hydrogen.

Action of Methyl Magnesium Iodide on Methylated Neprotine.—Methylated neprotine (0.0512 Gm.) gave, on similar treatment, 6.45 cc. of methane at normal temperature and pressure. $C_{17}H_{13}(NMe)(OMe)_4(OH)_2$ requires 5.7 cc. of methane at normal temperature and pressure, equivalent to two active hydrogen atoms.

Zinc Dust Distillation.—Neprotine hydrochloride (2 Gm.) was mixed with zinc dust (20 Gm.) and was heated in a current of hydrogen; the reaction product, which was brownish liquid, was absorbed in dilute hydrochloric acid (5%), and the mixture was slightly warmed. The content of the tube was extracted with ether and the ether solution was taken with the above hydrochloric acid solution. The ether layer yielded a tarry product which could not be characterized. The acid solution was basified with sodium hydroxide solution (5%) and then extracted with ether. The ether layer was washed with water and dried. The residue obtained on removal of ether was subjected to distillation at 70° and 0.3 mm. pressure to yield a very small quantity of practically colorless liquid which was identified as isoquinoline by the preparation of the following solid derivatives.

The Picrate.—A portion of the liquid was taken in dry ether and mixed with an ethereal solution of picric acid, and the mixture was kept overnight in a refrigerator, when a pale yellow-colored picrate separated, which was repeatedly crystallized from ethanol. The pure product, obtained as pale yellow needles, vaporized to a certain extent at 200° and finally melted at 215°. The picrate of an authentic specimen of isoquinoline melted similarly at 215°, after partial vaporization at 200°.

The Chloroplatinate.—The isoquinoline chloroplatinate was prepared in the usual way, and, on crystallization from water, shrank at 258° and melted with decomposition at 260–262°. Found: Pt. 27.9. Calcd. for $(C_9H_7N)_2$, H_2PtCl_6, Pt, 28.2%.

The Absorption Spectra of Neprotine Hydrochloride and Berberinium Chloride.—The ultraviolet absorption spectra of neprotine hydrochloride (0.05%) and of berberinium chloride (0.05%) solutions have been taken with an E_2 quartz spectrograph (Fig. 1). The source of continuous spectrum was an iron arc at 2.5 amperes and 220 volts d. c.

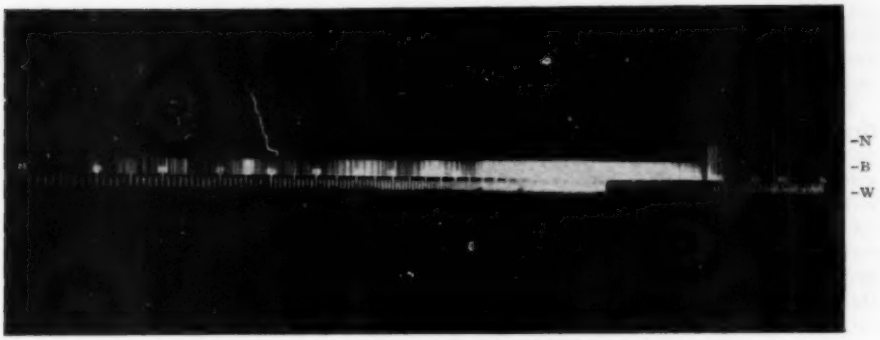


Fig. 1.—Absorption spectra.
N = neprotine hydrochloride; B = berberine chloride; and W = water.

Solution of 20-cm. length in the Baly's tube was placed on a stand just before the slit of the spectrograph, and was exposed to the arc for sixty seconds. In Fig. 1, the spectra No. 1 are due to the distilled water, No. 2 are due to berberinium chloride, and No. 3 are due to neprotine hydrochloride. The spectra No. 2 show a continuous absorption beginning from 4,700 Å. to the ultraviolet limit, and those of neprotine hydrochloride show the same type of continuous absorption but the limit of absorption is shifted to the longer wave length region 4,850 Å. This indicates that the absorption in both the solutions is due to the same essential central structure. The absorption in the visible region from 4,700 to 4,850 Å., in the case of neprotine hydrochloride, may be attributed to the difference in the functional groups of the molecule.

Oxidation of Neprotine with Manganese Dioxide and Sulfuric Acid.—Neprotine hydrochloride (2.5 Gm.) was treated with a mixture of sulfuric acid and water (2.5 cc. in 200 cc.), and stirred mechanically with manganese dioxide (2 Gm.) for one hour on a water bath. The mixture became brown and turbid. The reaction was slow and heating was continued for two additional hours. After cooling, the mixture was filtered. The brown-colored granular residue was dried in air and extracted with ether. On removal of ether no residue was left behind. The filtrate was then extracted exhaustively with ether. The ether was removed and the residue was sublimed as a white crystalline solid at 160° and 0.1 mm. pressure. The aqueous solution was made ammoniacal and exhausted with ether. The residue obtained on removal of ether was sticky and could not be worked up successfully.

Identification of Opianic Acid.—The white crystalline substance thus obtained was crystallized from water as fine colorless prisms, m. p. 146–147°, which did not depress the melting point of an authentic sample of opianic acid, m. p. 146–147°.

Oxime of Opianic Acid.—Opianic acid (1 mole) was dissolved in a dilute solution of potassium hydroxide (4 moles) mixed with hydroxylamine hydrochloride (1.5 moles) and left overnight. The mixture was then acidified with hydrochloric acid and allowed to stand, whereupon needle-shaped crystals separated which were crystallized twice from water m. p. 82°. Found: N, 6.1. Calcd. for $C_{10}H_{11}O_5N$, N, 6.2%.

Oxidation of Neprotine with Potassium Permanganate.—Neprotine hydrochloride (2 Gm.) was dissolved in warm water (60 cc.) and treated with potassium carbonate (0.5 Gm.) solution, and then oxidized by adding, in a slow stream, a solution of potassium permanganate (5%) until the pink color was not discharged. After standing for two hours, the mixture was filtered and the filtrate was decolorized by sulfur dioxide and evaporated to a small bulk (50 cc.). It was then acidified with hydrochloric acid extracted exhaustively with ether. The residue from the extract was dissolved in water and treated with calcium acetate to precipitate oxalic acid and filtered. The filtrate was acidified with hydrochloric acid and extracted again with ether. The residue from the ether solution was heated to remove acetic acid and then crystallized to a certain extent. It was sublimed in high vacuum, whereupon a crystalline solid separated which was converted into the ethylimide by heating with an excess of ethanolic ethylamine and evaporating to dryness. The product was sublimed *in vacuo* and, on crystallization from methanol, melted at 92°, either alone or in admixture with an authentic specimen of ethylimide of orthohemipinic acid. *Anal.*—Calcd. for $C_{10}H_9O_4N$, N, 5.9%. Found: N, 5.65.

Oxidation of Methylated Neprotine with Potassium Permanganate.—Methylated neprotine (2 Gm.) was likewise oxidized with alkaline potassium permanganate solution to yield a mixture of crystalline solids which were converted to the corresponding ethylimides by heating with an excess of ethanolic ethylamine and evaporating to dryness. The product was sublimed *in vacuo* and, on slow crystallization from methanol, separated in two fractions, m. p. 232° and 92°, respectively. The fractions seemed to contain two isomeric substances. Found for both samples: N, 5.6 and 5.69. Calcd. for $C_{10}H_9O_4N$, N, 5.9%. The product of m. p. 232° was therefore ethylimide of metahemipinic acid, and that of m. p. 92° was ethylimide of orthohemipinic acid.

The Molecular Formula of Neprotine.—A provisional formula $C_{19}H_{21}O_8N$. H_2O was suggested for neprotine (1). On the basis of the degradation experiments on neprotine, it was found that the base was more correctly represented by $C_{19}H_{23}O_8N$. The results of analyses of neprotine and its salts were found to agree with this modified formula. Found for neprotine: C, 60.46; H, 6.08; N, 3.91. Calcd. for $C_{19}H_{21}O_8N \cdot H_2O$, C, 60.48; H, 6.10; N, 3.71, and for $C_{19}H_{23}O_8N \cdot H_2O$, C, 60.16; H, 6.6; N, 3.69%. For neprotine hydrochloride, N, 3.5. Calcd. for $C_{19}H_{21}O_8N \cdot HCl$, N, 3.53, and for $C_{19}H_{23}O_8N.HCl$, N, 3.52%. For neprotine chloroplatinate, Pt, 17.58, 17.22. Calcd. for $(C_{19}H_{21}O_8N)_2$, H_2PtCl_6, Pt, 17.28, and for $(C_{19}H_{23}O_8N)_2$, H_2PtCl_6, Pt, 17.22%. For neprotine picrate, N, 9.7. Calcd. for $C_{25}H_{24}O_{12}N_4$, N, 9.52, and for $C_{25}H_{25}O_{13}N_4$, N, 9.49%.

SUMMARY

1. Neprotine is an alkaloid found in eight Indian species of *Mahonia* (Table I) examined so far. It is found that the roots of *M. borealis* and *M. Simonsii* are richest in this alkaloid, the per cent of yield being 0.31 and 0.27, respectively.

2. Neprotine is a secondary base and it contains two methoxyl, two phenolic hydroxyl, and two alcoholic hydroxyl groups.

3. On zinc dust distillation, it yields isoquinoline.

4. The ultraviolet absorption spectra of neprotine are similar to those of berberine.

5. On oxidation of neprotine with manganese dioxide and sulfuric acid, opianic acid is produced, and with potassium permanganate *ortho*-hemipinic acid.

6. Methylated neprotine on similar oxidation with potassium permanganate yields both *ortho*- and *meta*-hemipinic acids.

7. The structural formula of neprotine has been suggested to be (VI).

REFERENCES

(1) Chatterjee, R., THIS JOURNAL, **33**, 210(1944).
(2) Chatterjee, R., and Guha, M. P., *ibid.*, **39**, 181(1950).
(3) Chatterjee, R., and Guha, M. P., *ibid.*, **39**, 577(1950).
(4) Chatterjee, R., Guha, M. P., and Sen, S. K., *ibid.*, **40**, 36(1951).
(5) Chatterjee, R., and Guha, M. P., *ibid.*, **40**, 229(1951).
(6) Filippo, J. D., *Arch. Pharm.*, **236**, 601(1898).
(7) Ghose, T. P., Krishna, S., and Schlittler, E., *Helv. Chim. Acta*, **17**, 919(1934).
(8) Clowes, G. H. A., and Tollens, B., *Ber.*, **32**, 2841 (1899).
(9) Zeisel, S., *Monatsh.*, **6**, 989(1886).
(10) Zerewitinoff, T., *Ber.*, **45**, 2384(1912).
(11) Beckett, G. H., and Wright, C. R. A., *J. Chem. Soc.*, **28**, 573(1875).
(12) Chatterjee, R., THIS JOURNAL, **38**, 149(1949).
(13) Spaeth, E., and Dobrowsky, A., *Ber.*, **58**, 1275(1925).
(14) Manske, R. H. F., *Can. J. Research*, **17**, 51(1939).
(15) Mahala, B., *Silliman's Amer. J.*, **33**, 43(1862).
(16) Gurguel, L., *et al.*, *Bull. Assoc. Brasil Pharm.*, **15**, 11 (1934).
(17) Haworth, R. D., and Perkin, W. H., *J. Chem. Soc.*, **127**, 1453(1925).
(18) Girardet, A., *Helv. Chim. Acta*, **14**, 504(1931).

The Ultraviolet Determination of Aureomycin and Terramycin*

By DOROTHY J. HISCOX†

The ultraviolet absorption curves of aureomycin hydrochloride and terramycin hydrochloride in different solvents are presented. Methods for the ultraviolet determination of aureomycin and terramycin based upon the acid hydrolysis of these antibiotics are outlined. Results by these methods are compared with bioassay results.

IN A PREVIOUS issue of THIS JOURNAL, aureomycin and terramycin were compared with respect to their bacterial spectrum, serum concentration, and excretion (1). During an investigation of the ultraviolet absorption of these antibiotics, some interesting comparisons in their properties were noted. It is the purpose of this paper to present for comparison the ultraviolet absorption spectra of aureomycin and terramycin and methods of analysis based upon them.

All absorption spectra were obtained using a Beckman DU Quartz Spectrophotometer. In most cases readings were made at intervals of 2.5 mμ from 220 to 400 mμ, but in some instances the interval was reduced to 1 mμ.

AUREOMYCIN

The absorption spectrum of aureomycin hydrochloride in 0.1 *N* hydrochloric or sulfuric acid shows maxima at 230, 262.5, and 367.5 mμ (2), with minima at 240 and 305 mμ. When 0.1 *N* sodium hydroxide is used as the solvent, the maxima appear at

* Received December 5, 1950, from the Laboratory of Hygiene, Department of National Health and Welfare, Ottawa, Canada.

† Thanks are due to Miss K. Fitzpatrick who made the bioassay determinations.

255, 285, and 345 mμ (2), with the minima at 237.5, 267.5, and 320 mμ. In aqueous solution the absorption spectrum has maxima at 230, 275, and 367.5 mμ, and minima at 257.5 and 327.5 mμ. These absorption curves are shown in Fig. 1. When solutions of aureomycin hydrochloride in 0.1 *N* hydrochloric acid or water are refrigerated, there is no change in their absorption spectra over a period of a week. A refrigerated alkaline solution shows a daily change in absorption until, at the end of two weeks, the maxima are at 255 and 317.5 mμ and the minima at 237.5 and 292.5 mμ. This curve is also included in Fig. 1.

It is possible to estimate aureomycin quantitatively in preparations of high purity simply by making proper dilutions in 0.1 *N* hydrochloric acid and measuring the absorbance of the solution at 262.5 and 305 mμ. The difference in absorbance is proportional to the amount of aureomycin present. Similarly, it may be determined in 0.1 *N* sodium hydroxide by taking immediate readings at 285 and 320 mμ. From the curves in Fig. 1 it is obvious that readings at other wave lengths may also be used.

When a solution of aureomycin hydrochloride in 0.1 *N* acid is stored at 4° for two weeks, no change occurs in the ultraviolet absorption spectrum. In 1 *N* sulfuric acid the antibiotic has the same original absorption as in 0.1 *N*, but on standing at room temperature a gradual change occurs over a period of weeks. The maxima at 230 and 262.5 first become more pronounced, then gradually subside, while that at 367.5 decreases steadily. When a solution in 1 *N* sulfuric acid is refluxed, pronounced changes occur in the absorption spectrum. After thirty minutes, maxima have appeared at 250 and 270 mμ. As the refluxing time is increased, the maximum at 270 mμ gradually decreases and the one at 250 mμ becomes more pronounced until, after four hours, only a suggestion of the one at 270 mμ remains. The four-hour absorption curve is shown in Fig. 2.

If, instead of refluxing, the solution of aureomycin hydrochloride in 1 *N* sulfuric acid is heated in boiling water, it can be shown that the maximum at the longer wave length is the first to appear, and does so rapidly. Figure 2 shows the absorption curves of such solutions which have been heated for five and fifteen minutes. The five-minute curve shows a pronounced peak at 274 mμ. The ten-minute curve is identical. After fifteen minutes of heating, the peak at 274 mμ has begun to subside and there is slight indication of absorption at 250 mμ. The formation of the maximum at 274 mμ has been used as the basis of the method presented in this paper for the determination of aureomycin. Treatment with acid at 100° has been used for the colorimetric determination of aureomycin (3).

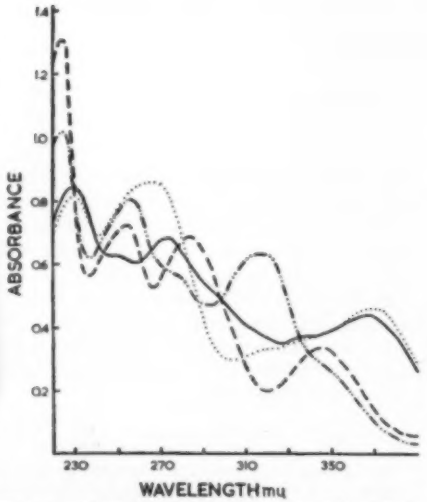


Fig. 1.—Aureomycin hydrochloride 25 μg./ml. —, H_2O; . . . . . , 0.1 *N* H_2SO_4; – –, 0.1 *N* NaOH; – · – · – , 0.1 *N* NaOH two weeks at 4°.

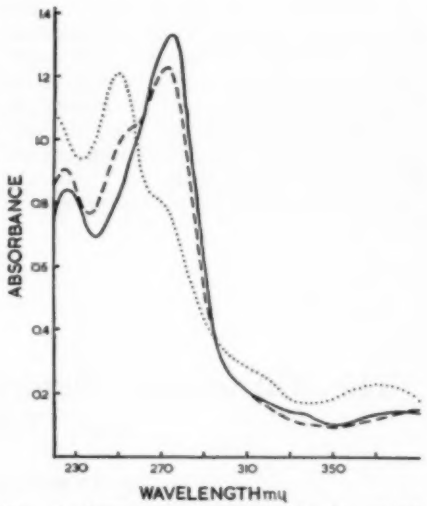


Fig. 2.—Aureomycin hydrochloride 20 μg./ml. 0.4 *N* H_2SO_4.

Heated at 100° in *N* H_2SO_4 for five minutes, —; for fifteen minutes, – – –. Refluxed in *N* H_2SO_4 for four hours, ...

TERRAMYCIN

In 0.1 *M* phosphate buffer at *p*H 4.5, terramycin shows absorption maxima at 249, 276, and 353 mμ (4). Almost the same maxima are exhibited by an aqueous solution of the hydrochloride. In 0.1 *N* sodium hydroxide, maxima are found at 245, 267, and 375 mμ, with a pronounced minimum at 320 mμ. In 0.1 *N* sulfuric acid the maxima appear at 267 and 355 mμ with a minimum at 300 mμ. The absorption curves are shown in Fig. 3. Like aureomycin, in samples of high purity terramycin may be determined in 0.1 *N* sodium hydroxide by measuring the absorbance of the solution at 265 and 320 mμ immediately, and in 0.1 *N* sulfuric acid by taking readings at 267 and 300 mμ.

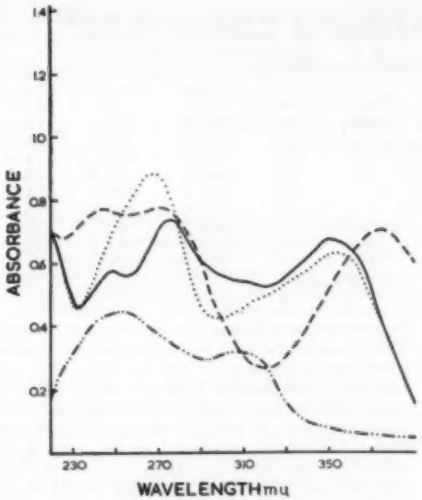


Fig. 3.—Terramycin hydrochloride 20 μg./ml. —, H_2O; . . . . . 0.1 *N* H_2SO_4; – – –, 0.1 *N* NaOH; – • – • – , 10 μg./ml. 0.1 *N* NaOH, two weeks at room temperature.

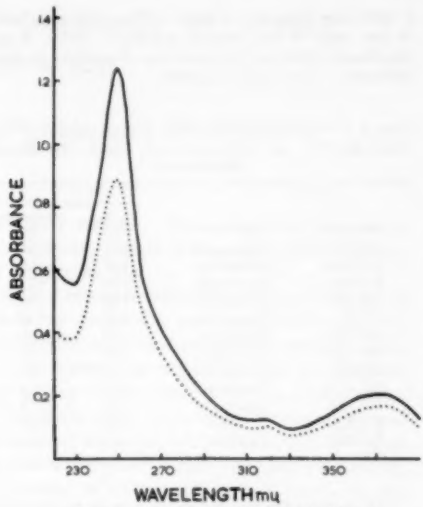


Fig. 4.—Terramycin hydrochloride. —, refluxed four hours in 0.1 *N* H_2SO_4, 10 μg./ml. 0.05 *N* H_2SO_4; . . . . . , heated at 100° for thirty minutes in 0.5 *N* H_2SO_4, 8 μg./ml. 0.12 *N* H_2SO_4.

On standing at room temperature there is no change in the absorption spectrum of the aqueous solution for three days, or of the acid solution for one week. Like aureomycin, the alkaline solution of terramycin hydrochloride shows immediate change in its ultraviolet absorption. In twenty-four hours the maximum at 375 mμ has disappeared and new ones have formed at 250 and 305 mμ. After two weeks the maximum at 250 has broadened somewhat and the peak shifted to 255 mμ. The two-week absorption curve is included in Fig. 3.

When terramycin hydrochloride is refluxed in 0.1 *N* sulfuric acid, its ultraviolet absorption spectrum is changed. A pronounced maximum appears at 249 mμ. Figure 4 shows the curve of a solution that has been refluxed for four hours. In 0.5 *N* sulfuric the antibiotic gives a similar curve if heated in boiling water, as shown by the dotted line in Fig. 4. Unlike aureomycin, the maximum does not show its greatest absorbance until the solution has been heated for thirty minutes. Increasing the strength of the acid does not increase the extent of the maximum nor decrease the heating time required. This maximum is used for the determination of terramycin outlined in this paper.

METHODS

For the determination of aureomycin the following method has proved satisfactory:

To a 5-ml. aqueous aliquot containing 100 to 500 μg. of aureomycin in a 25 × 200-mm. test tube, add 5 ml. 2 *N* H_2SO_4. Heat in boiling water for eight minutes. Cool. Transfer to a 25-ml. volumetric flask and make to volume with H_2O. Measure the absorbance of the solution at 274 and 350 mμ. The difference in absorbance is proportional to the aureomycin present in solution.

With 63 aliquots containing from 10 to 600 μg. of aureomycin, a regression line was established using this method. The equation for this line was $Y = 15.427258\ X + 0.1236$, where Y is the μg. of aureomycin per ml. of the final solution and X the difference in absorbance. The correlation coefficient was 0.9997.

Terramycin may be readily determined as follows:

To a 3-ml. aqueous aliquot containing 25–250 μg. of terramycin in a 25 × 200-mm. test tube, add 3 ml. *N* H_2SO_4. Insert a foil-wrapped stopper through which a 12-in. piece of glass tubing has been passed to serve as an air condenser. Heat in boiling water for thirty minutes. Cool. Transfer to a 25-ml. volumetric flask and make to volume with H_2O. Determine the absorbance of the solution at 249 and 312 mμ. The terramycin present is proportional to the difference in absorbance.

A regression line was established for the determination of terramycin using 85 aliquots containing from 10 to 300 μg. The equation of the line was $Y = 9.407564\ X + 0.0272$, where X is the difference in absorbance and Y the μg. of terramycin per ml. of the final solution. The correlation coefficient was 0.9918.

RESULTS

Aureomycin capsules, troches, and ointments have been analyzed using the above method. The base used for the troches interfered with the determination so the troches were powdered, then shaken with 95% ethyl alcohol to dissolve the aureomycin. Before analysis the extract was diluted with four times its volume of water. The ointments were dissolved in ether and the aureomycin extracted into water. The results are given in Table

I with the bioassay results. They are expressed as per cent of the labeled potency. There is no significant difference between the results by the two methods. The t value is 0.697.

TABLE I.—RESULTS FOR THE DETERMINATION OF AUREOMYCIN BY ULTRAVIOLET AND BIOASSAY METHODS

Sample	Nature	Results U. V., %	Results Bioassay, %
50212	Ointment	101	104
501594	Ointment	107	113
501623	Ointment	110	117
501800	Ointment	112	105
50545	Capsules	106	100
501595	Capsules	112	105
501698	Capsules	100	100
501801	Capsules	111	104
501075	Troches	115	103
501315	Troches	112	111
501339	Troches	103	105
501799	Troches	108	114

The method for the determination of terramycin has been used to analyze terramycin capsules. These results and those by the bioassay are presented in Table II. The t value of 1.140 indicates that there is no significant difference between the methods.

TABLE II.—RESULTS FOR THE DETERMINATION OF TERRAMYCIN BY THE ULTRAVIOLET AND BIOASSAY METHODS

Sample	Potency, Mg.	Results U. V., %	Results Bioassay, %
501904	50	100	96
501922	50	95	98
501921	100	109	95
501889	100	109	97
501888	250	100	100
501903	250	99	104

It should be noted that the ultraviolet and bioassay determinations were made on different solutions of the samples. This fact may be responsible for part of the difference between the two methods. This is especially true in the analysis of capsules where the variation in fill may be large. The contents of several capsules from two bottles of aureomycin capsules were weighed, then analyzed. Capsules containing 50 and 250 mg. aureomycin were used. The results, expressed as per cent of the labeled potency, and the weights are listed in Table III, together with the ratio of the weight to the result. It is obvious that the difference in per cent is proportional to the variation in the weight of the capsule contents.

TABLE III.—COMPARISON OF THE WEIGHT OF THE CONTENTS OF AUREOMYCIN CAPSULES AND THE RESULTS OF U.V. ANALYSIS

Sample	Capsule	Weight, Mg.	Results, %	Wt./+%
50303	1	171.5	98.0	175.0
	2	173.7	99.2	175.1
	3	180.5	103.2	174.9
	4	165.0	94.0	175.5
	5	191.7	109.6	174.9
	6	179.6	102.6	175.0
50302	1	499.8	95.2	525.0
	2	578.7	110.2	525.1
	3	582.5	111.0	524.8

SUMMARY

Methods for the determination of aureomycin and terramycin in preparations of these compounds have been presented. These methods are based upon the ultraviolet absorption spectra of the antibiotics after treatment with dilute sulfuric acid at 100°. There is no significant difference between results by these ultraviolet methods and those obtained by the bioassay.

REFERENCES

(1) Welch, H., Hendricks, F. D., Price, C. W., and Randall, W. A., THIS JOURNAL, 39, 185(1950).
(2) Broschard, R. W., Dornbush, A. C., Gordon, S., Hutchings, B. L., Kohler, A. R., Krupka, G., Kushner, S., Lefemine, D. V., and Peducks, C., *Science*, 109, 199(1949).
(3) Levine, J., Garlock, E. A., Jr., and Fischbach, H., THIS JOURNAL, 38, 473(1949).
(4) Regna, P. P., and Solomons, I. A., *Ann. N. Y. acad. Sci.*, 53, 229(1950).

Terramycin: Chemical Methods of Assay and Identification*

By F. MONASTERO, JOHN A. MEANS, T. C. GRENFELL, and F. HOWARD HEDGER†

Two colorimetric methods and one spectrophotometric method for the chemical assay of terramycin are presented. Their range of application and comparisons with microbiological assays are given, along with qualitative tests for the identification of terramycin.

TERRAMYCIN (1, 2), a new broad spectrum antibiotic obtained from cultures of *Streptomyces rimosus*, has become an important chemotherapeutic agent in medical practice. In support of the chemical, pharmacological, and clinical studies on this new antibiotic, accurate and rapid methods of assaying terramycin have been developed. In biological systems, where terramycin is found in minute amounts in the presence of many other entities, the microbiological method described by Kersey (3) has been found to be quite reliable. For the determination of terramycin in various dosage forms and for a broad range of concentrations in many biological systems, chemical assays have been found to be suitable with respect to reproducibility, ease of operation, and speed. In this paper the simplest and most efficient chemical assay methods devised in our laboratory are presented.

Many of the basic chemical and physical properties of terramycin have been described by Finlay, *et al.* (1), and Regna and Solomons (4). Among the properties which might serve as a basis for the determination of terramycin, the following have been found to be the most suitable: (*a*) the color produced by ferric chloride in dilute acidic solutions of terramycin; (*b*) the disappearance of a characteristic band in the ultraviolet spectrum of terramycin by the action of dilute alkali; and (*c*) the color produced by the alkaline degradation products of terramycin. Of the three methods based on these properties of terramycin, the colorimetric ferric chloride method has been most widely used. The primary standard used in our analytical work is a pure terramycin base dihydrate ($C_{22}H_{24-28}N_2O_8 \cdot 2H_2O$), 1 mg. of which is equivalent to 925 μg. of anhydrous base. One milligram of pure terramycin hydrochloride is also equivalent to 925 μg. of anhydrous base.

Other methods which have been found to be useful as qualitative and quantitative tests for terramycin are based on optical rotation, infrared absorption, the bright red color produced by the action of concentrated sulfuric acid, and the color of the coupling product of terramycin and diazotized sulfanilic acid. The specificity of all the tests described in this paper has not been investigated to the extent that they may be recommended for the determination of terramycin in certain biological systems such as body fluids. The diazotization reaction is now being tested as a possible alternative to the microbiological method for pharmacological and clinical work.

COLORIMETRIC FERRIC CHLORIDE METHOD

The absorption curve of the orange-brown color developed in a typical terramycin solution indicated strong adsorption in the blue region of the spectrum, and experimentation with various filter systems showed a 490 mμ filter to give the desired results. Subsequent collection of data established the validity of the Beer-Lambert law over the concentration range finally used. The following procedure has been used successfully in our laboratories.

Method

Prepare a solution that contains approximately 0.5 mg. of terramycin per ml. and has a *p*H of 2. Transfer a 1.0-cc. or a 2.0-cc. aliquot to a test tube and dilute to 10.0 cc. with 0.01 *N* HCl. Add 10.0 cc. of 0.05% ferric chloride in 0.01 *N* HCl and mix. Allow the test tube to stand at room temperature for ten minutes. A photoelectric colorimeter with a 490 mμ filter is set for 100% transmittancy with a reagent blank prepared similarly and at the same time. The reagent blank is replaced with the unknown solution, and the per cent transmittancy recorded. The concentration of the unknown solution is determined by referring to the standard curve made by reading the color developed in aliquot portions of a solution containing 500 μg./cc. of terramycin in 0.01 *N* HCl. The *p*H of this solution should be approximately 2. The standard curve shown in Fig. 1 demonstrates the adherence to the Beer-Lambert law when appropriate aliquots are treated and read as above. The color is stable for at least two hours.

The samples shown in Table I are but a few of the many types assayed, and all the results show

* Received January 30, 1951, from the Analytical Laboratories, Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

† The authors wish to express their thanks to Mr. Kane, Dr. Lazier, Dr. Brunings, and Mr. Stone for their comments and constructive criticisms of this paper.

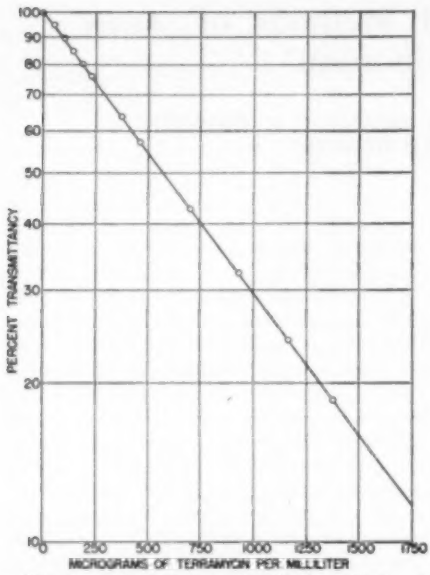


Fig. 1.—Colorimetric ferric chloride method. A standard curve showing the relationship of terramycin concentration to transmittancy using a 490 mμ filter

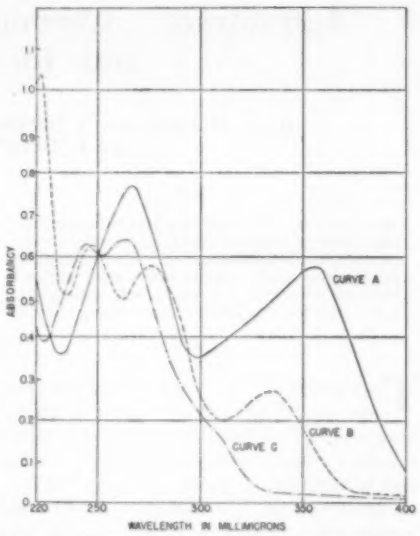


Fig. 2.—Ultraviolet absorption spectra of terramycin.

Curve *A*—Untreated in 0.1 *N* HCl. Curve *B*—Caustic-treated in alkaline solution. Curve *C*—Caustic-treated in 0.1 *N* HCl.

good agreement between chemical and microbiological assay. Fermentation broths have also been assayed successfully. Application of the method to other fields, such as in clinical investigations, has not been fully explored.

A series of several hundred samples, run in duplicate, established that the method is easily reproducible within ±2%. By using appropriate aliquots, the method is applicable to solutions containing as low as 50 μg. terramycin per cc.

The appearance of a complete olive-green color ten minutes after the addition of the reagent denotes degradation. Such samples show very low biological activity, and cannot be estimated colorimetrically.

Interference from insoluble or immiscible material found in the make-up of capsules, tablets, and ointments may be eliminated by filtering, centrifuging, or extracting, with practically no loss of the active component.

SPECTROPHOTOMETRIC METHOD

The ultraviolet absorption spectrum of crystalline terramycin dihydrate in dilute acid (*p*H 1.7) has the following maxima $E^{1\%}_{1\ cm.}$ 353 mμ = 277 $E^{1\%}_{1\ cm.}$ 268 mμ = 379 (Fig. 2, curve *A*). After boiling for five minutes in 0.2 *N* NaOH, the characteristic absorption spectrum changes with a sharp visual increase in yellow-orange coloration (Fig. 2, curve *B*). Such a caustic-treated terramycin solution when acidified to *p*H 1.7 shows no maximum at 353 mμ (Fig. 2, curve *C*). The differential absorption measured at 353 mμ can be used for the spectrophotometric assay of terramycin. The change from yellow to a yellow-orange coloration upon caustic treatment has been found to be specific for terramycin relative to the other common antibiotics and has been made the basis for a quantitative method of assay for terramycin.

TABLE I.—COMPARISON OF COLORIMETRIC (IRON) AND MICROBIOLOGICAL ASSAYS

Sample	Microbiological	Colorimetric
Powder	900 μg/mg.	915 μg/mg.
Capsules (250 mg.)	255 mg./cap.	253 mg./cap.
Capsules (100 mg.)	98 mg./cap.	101 mg./cap.
Capsules (50 mg.)	50 mg./cap.	50 mg./cap.
Ointment-topical (30 mg./Gm.)	32 mg./Gm.	31 mg./Gm.
Ointment-ophthalmic (1 mg./Gm.)	1.05 mg./Gm.	1.1 mg./Gm.
Troches (15 mg.)	15.5 mg./troche	15.3 mg./Gm.
Intravenous powder (500 mg./vial)	500 mg./vial	507 mg./vial
Ophthalmic powder (25 mg./vial)	25.8 mg./vial	26.2 mg./vial
Tablets (250 mg.)	265 mg./tablet	255 mg./tablet
Elixir (50 mg./cc.)	50 mg./cc.	50 mg./cc.

Method

Prepare a solution containing approximately 0.5 mg. of terramycin per cc. in 0.01 *N* HCl. Transfer a 2.0-cc. aliquot to a test tube and dilute to 5 cc. with water. Add 5 cc. of 0.4 *N* NaOH and immerse in a boiling water bath for five minutes and then cool for two minutes in ice water. Dilute to exactly 20 cc. with water. This solution may be used directly in the colorimetric method.

Prepare and read the absorbancy of the following solutions at 353 mμ in a spectrophotometer.

Solution A.—Dilute 10 cc. of the original solution containing 0.5 mg./cc. of terramycin to 250 cc. with 0.1 *N* HCl.

Solution B.—Dilute 10 cc. of final caustic-treated solution to 25 cc. with 0.17 *N* HCl.

The difference in absorbance (solution A − solution B) is a direct measure of the concentration of terramycin which can be read from a graph prepared using terramycin of known potency (Fig. 3). This method has been applied to terramycin in its various dosage forms, as well as to all control assays, with excellent results, as shown in Table VI. This method is applicable to solutions of terramycin in the range of 15 μg./cc. when more concentrated reagents are used in the above method.

CAUSTIC DEGRADATION COLORIMETRIC METHOD

The per cent transmittancy of the original alkaline solution used in the spectrophotometric method is measured at 440 mμ in a colorimeter with a water blank. By plotting the per cent transmittancy against concentration on semilog paper, a straight line is obtained (Fig. 4).

The effect of caustic concentration is shown in Table II, which indicates the per cent transmittancy of solutions containing 2.3 mg. of terramycin after heating with varying concentrations of alkali in the same manner as outlined in the procedure. While minor variations in alkalinity have no sharp effect on color concentration, care in alkali addition is necessary for reproducibility.

TABLE II.—EFFECT OF CAUSTIC CONCENTRATION VARIATIONS ON COLOR IN CAUSTIC ASSAY USING 2.3 MG. OF TERRAMYCIN

Sodium Hydroxide Concentration, *N*	Transmittancy, %
0.02	27.5
0.04	24.5
0.05	25.0
0.10	28.5
0.15	34.5
0.20	34.5
0.25	38.0
0.50	45.0
1.00	52.0

The effect of variations in heating time at a constant alkalinity (0.2 *N* NaOH) is shown in Table III. While insensitive to minor time interval changes, a consistent heating time is recommended.

Complete destruction of the 353 mμ absorption peak of terramycin does not occur in the shorter

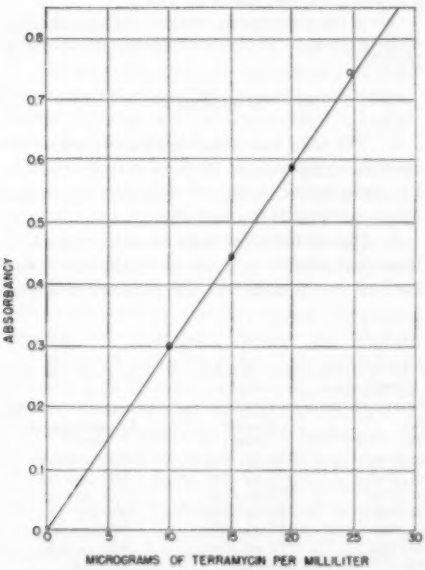


Fig. 3.—Spectrophotometric method. A standard curve showing the relationship between terramycin concentration and absorbance difference between caustic-treated and untreated terramycin solutions both in 0.1 *N* HCl at 353 mμ.

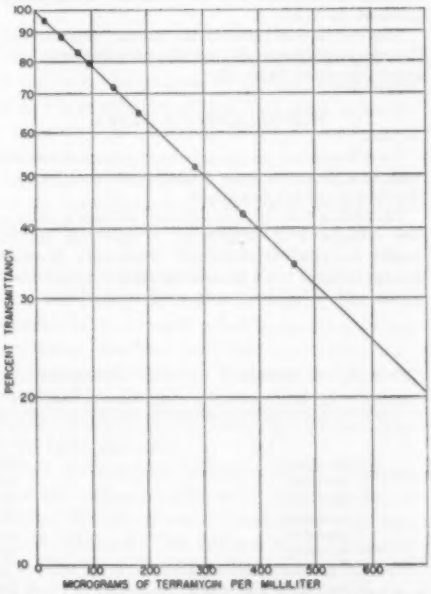


Fig. 4.—Caustic degradation colorimetric method. A standard curve showing the relationship of terramycin concentration to transmittancy at 440 mμ.

TABLE III.—EFFECT OF HEATING TIME VARIATIONS ON COLOR IN CAUSTIC ASSAY USING 2.3 MG. OF TERRAMYCIN

Time, Min.	Transmittancy, %
1	30.0
2	31.0
3	34.5
4	34.3
5	35.0
7	34.8
10	34.7

heating intervals, with the result that the color measured in a colorimeter does not follow the Beer-Lambert law. Interferences by other antibiotic preparations have not been sufficient to destroy the specificity of the test. The stability of the resultant color is shown in Table IV.

TABLE IV.—RESULTANT COLOR STABILITY, USING 2.3 MG. OF TERRAMYCIN IN THE CAUSTIC ASSAY

Time Standing, Min.	Transmittancy, %
5	34.7
10	34.7
15	34.7
20	34.7
30	35.0
60	35.0
90	35.5
120	35.5

A comparison of the results obtained using the colorimetric method and the microbiological assay is shown in Table V.

The correlation between the caustic degradation, the spectrophotometric and the microbiological assays is shown in Table VI.

MISCELLANEOUS TESTS

To differentiate terramycin from other antibiotics, tests in addition to those described above have been developed and are listed here.

On adding 2 cc. of concentrated sulfuric acid to a few milligrams of terramycin, a relatively stable, bright red color is produced. This color reaction has been found to be specific for terramycin as compared with other commonly used antibiotics.

TABLE VI.—COMPARISON OF COLORIMETRIC, SPECTROPHOTOMETRIC, AND MICROBIOLOGICAL ASSAYS OF TERRAMYCIN HYDROCHLORIDE

Sample	Colorimetric (Caustic), μg./Mg.	Spectrophotometric, μg./Mg.	Microbiological, μg./Mg.
A	910	900	895
B	900	920	895
C	910	900	895
D	915	905	900
E	910	920	890
F	915	920	905
G	910	920	890
H	905	915	905
I	915	900	890
J	915	900	895
K	920	915	895
L	900	920	880
M	920	900	890

Terramycin couples with various diazotizing agents to give colored compounds. For example, when a solution of diazotized sulfanilic acid is added to a solution of terramycin, a stable, intense, orange-red color is produced. This reaction is now being studied as another possible colorimetric method for the determination of terramycin, and quantitative results can be obtained by measuring the color produced at 490 mμ.

The specific rotation of terramycin in 0.1 *N* HCl is $[\alpha]_D^{25} = -196°$ (C = 0.5%). The specific rotation of terramycin base in methanol is $[\alpha]_D^{25} = +26°$ (C = 0.5%). The difference in optical rotation in these two solvents is specific for terramycin. The optical rotation of terramycin in 0.1 *N* HCl is a quantitative estimation of its purity.

One of the most specific tests for the identification of terramycin is its infrared absorption spectrum (2, 4).

SUMMARY

1. The color reaction of terramycin with ferric chloride in dilute acid solution may be used for the colorimetric assay of solutions containing 50 μg. terramycin/cc. or higher.

2. One of the ultraviolet absorption peaks is destroyed when a solution of terramycin undergoes caustic degradation and subsequent acidifi-

TABLE V.—COMPARISON OF TWO COLORIMETRIC AND MICROBIOLOGICAL ASSAYS OF TERRAMYCIN HCl POWDER AND CAPSULES

Lot	Iron Assay	Caustic Assay	Microbiological Assay
1 (powder)	920 μg./mg.	910 μg./mg.	890 μg./mg.
2 (powder)	920 μg./mg.	900 μg./mg.	890 μg./mg.
3 (powder)	905 μg./mg.	905 μg./mg.	900 μg./mg.
4 (powder)	905 μg./mg.	915 μg./mg.	900 μg./mg.
5 (powder)	905 μg./mg.	910 μg./mg.	880 μg./mg.
6 (powder)	910 μg./mg.	915 μg./mg.	890 μg./mg.
7 (capsules) (250 mg.)	258 mg.	258 mg.	250 mg.
8 (capsules) (250 mg.)	248 mg.	248 mg.	248 mg.
9 (capsules) (250 mg.)	245 mg.	245 mg.	245 mg.
10 (capsules) (250 mg.)	260 mg.	255 mg.	252 mg.
11 (capsules) (50 mg.)	50 mg.	49 mg.	50 mg.

cation. This has been made the basis of a spectrophotometric method applicable to solutions containing 15 μg/cc. or more of terramycin.

3. The color formed on alkaline degradation has been used as the basis of a colorimetric method applicable to solutions of about 200 μg./cc.

4. Comparative results between the microbiological assay and the above methods are given.

5. Identification tests for terramycin are described.

REFERENCES

(1) Finlay, A. C., Hobby, G. L., P'an, S. Y., Regna, P. P., Routien, J. B., Seeley, D. B., Shull, G. M., Sobin, B. A., Solomons, I. A., Vinson, J. W., and Kane, J. H., *Science*, 111, 85(1950).
(2) Sobin, B. A., Finlay, A. C., and Kane, J. H., U. S. pat. 2,516,080, (to Chas. Pfizer & Co., Inc.), July 18, 1950.
(3) Kersey, R. C., THIS JOURNAL, 39, 252(1950).
(4) Regna, P. P., and Solomons, I. A., *Ann. N. Y. Acad. Sci.*, 53, 229(1950).

The Determination of Choline in the Presence of B Complex Vitamins*

By F. J. BANDELIN and J. V. TUSCHHOFF

Interference by various members of the vitamin B complex in the reineckate method for the determination of choline may be overcome by precipitation of choline at controlled *p*H levels. Thiamine, nicotinic acid, nicotinamide, and pyridoxine, which ordinarily produce precipitates with ammonium reineckate reagent, are not precipitated in alkaline solution from which choline may be separated as the insoluble reineckate. This is utilized as a basis for separation and the subsequent colorimetric determination of choline. Data are given showing the effect of increasing *p*H on the precipitation of the interfering B vitamins and on choline by ammonium reineckate reagent, and on the recovery of choline from mixtures of the various vitamins of the B complex.

THE ROLE of choline as an essential metabolite and its relationship to the vitamin B complex suggests the possibility of numerous pharmaceutical mixtures for the concomitant administration of these compounds.

The reineckate method for the determination of choline in the presence of vitamins of the B complex is complicated by the fact that thiamine, pyridoxine, nicotinic acid, and nicotinamide also yield insoluble reineckates. Riboflavin and pantothenic acid do not interfere.

Although numerous procedures and techniques involving the reineckate method for choline have appeared in the literature (1–5), these have generally dealt with the isolation of choline from plant or animal tissues, in which choline exists largely as phospholipids, and the hydrolysis of the extraction products to liberate free choline prior to its precipitation as the reineckate. In most natural substances the ratio of choline to other vitamins of the B complex is so great as to make interference by these compounds an almost negligible factor.

* Received July 11, 1950, from the Research Laboratories of Flint, Eaton & Co., Decatur, Ill.

In this paper, a method for the determination of choline in the presence of significant quantities of synthetic vitamins of the B complex generally encountered in pharmaceutical preparations is described.

Preliminary experiments indicated that under certain conditions, which are later described in detail, choline could be quantitatively precipitated as the reineckate from mixtures of other factors of the vitamin B complex. This is based upon the ability of choline to form an insoluble reineckate in strongly alkaline solution, while pyridoxine, nicotinic acid, and nicotinamide do not possess this ability. Thiamine is rapidly decomposed in alkaline solution and its decomposition products do not yield reineckates at relatively high *p*H levels.

This investigation was undertaken to determine the precise conditions for the separation of choline through selective precipitation at controlled *p*H levels. To this end the precipitation of choline from alkaline buffers ranging from *p*H 7.0 to 11.0, inclusive, at 1.0 *p*H unit increments, both alone and in the presence of varying amounts of other B complex factors, was studied.

METHOD

Reagents

Ammonium Reineckate Reagent.—Ammonium reineckate (2%) in anhydrous methanol.

Wash Water.—Distilled water containing 2 ml. of ammonium reineckate reagent per liter.

Acetone.—Reagent grade.

Standard Solution of Choline Chloride.—A solution containing 1 mg. of choline chloride per ml. (Choline chloride should be dried over magnesium perchlorate or anhydrous calcium sulfate in a vacuum desiccator.)

Buffers:[1]

Buffer *p*H 7.0[2]
Buffer *p*H 8.0[2]
Buffer *p*H 9.0[2]
Buffer *p*H 10.0[2]
Buffer *p*H 11.0[3]

Trisodium phosphate, reagent grade, 10% solution.

Procedure.—To 5 ml. of each buffer, 5 ml. (5 mg.) of choline chloride was added and mixed. Two milliliters of ammonium reineckate reagent was then added and the mixture allowed to stand for thirty minutes. The choline reineckate was collected by filtering through a 15-ml. low-form sintered glass crucible of medium porosity with the aid of gentle suction. The precipitate was washed with two 5-ml. portions of wash water and sucked dry.

The choline reineckate was then dissolved in acetone by placing the crucible in a glass funnel with a suitable rubber adapter. The stem of the funnel is drawn into a capillary having a diameter of 1–2 mm. so that it fits into the neck of a 25-ml. volumetric flask with the end of the capillary just above the 25-ml. mark. Portions (5 ml. each) of acetone are pipetted into the crucible and forced through by air pressure exerted upon the surface. This is accomplished by placing a one-hole rubber stopper which just fits into the top of the crucible. The end of a glass tube equipped with a rubber bulb at the opposite end is passed through the hole in the rubber stopper and air pressure in the crucible increased by gentle pressure on the rubber bulb. This forces the acetone through the sintered glass, carrying the choline reineckate in solution with it.

Transmittance of the acetone solution of choline reineckate was determined spectrophotometrically at 525 mμ.

EXPERIMENTAL

To determine the effect of increasing alkalinity upon the precipitation of choline as the reineckate, 5-mg. aliquots of choline chloride were subjected to determinations, as described, in the various alkaline buffers from *p*H 7.0 to 11.0. The results are shown in Table I.

Since alkalinity of buffers up to *p*H 11.0 seems to have no appreciable effect upon the precipitation of choline reineckate, further investigation of the precipitation of choline reineckate in the presence of other vitamins of the B complex was carried out in buffer *p*H 11.0. This buffer was chosen because the other B complex factors involved, except thiamine under certain conditions, produced no precipitate with ammonium reineckate solution at this *p*H. This is demonstrated in Table II in which other vitamins of the B complex as listed, at various levels of concentration, were treated with ammonium reineckate reagent, at increasing *p*H levels at one-unit increments from *p*H 7.0 to 11.0, as shown.

As shown in Table II, only choline and thiamine produced precipitates with ammonium reineckate at *p*H levels above 7.0. In the case of thiamine, lower concentrations (5 and 10 mg.) did not produce a precipitate at *p*H 11.0. This was due to rapid decomposition of the thiamine in the alkaline solution. Higher concentrations (25 and 50 mg.) produced precipitates at this *p*H level if the reagent was added soon after the thiamine was mixed with the alkaline buffer. If the thiamine was allowed to stand in the alkaline buffer for any length of time, or if the solution was heated to 50° in a water bath, no precipitation occurred upon the addition of ammonium reineckate reagent. It is apparent from this that the decomposition of products of thiamine do not yield insoluble reineckates at elevated *p*H values.

TABLE I.—THE PRECIPITATION OF CHOLINE REINECKATE AT VARIOUS *p*H LEVELS

Sample, Mg.		Found, Mg.	Recovery, %
Precipitation in *p*H 7.0 Buffer			
5.00		5.02	100.4
5.00		5.10	102.0
5.00		4.98	99.6
5.00		4.97	99.4
5.00		4.99	99.8
	Av.	5.01	100.2
Precipitation in *p*H 8.0 Buffer			
5.00		4.99	99.8
5.00		4.96	99.2
5.00		5.03	100.6
5.00		4.95	99.0
5.00		4.98	99.6
	Av.	4.98	99.6
Precipitation in *p*H 9.0 Buffer			
5.00		5.00	100.0
5.00		4.94	98.8
5.00		5.02	100.4
5.00		4.92	98.4
5.00		4.96	99.2
	Av.	4.97	99.4
Precipitation in *p*H 10.0 Buffer			
5.00		5.02	100.4
5.00		4.99	99.8
5.00		5.07	101.4
5.00		4.97	99.4
5.00		4.99	99.8
	Av.	5.01	100.2
Precipitation in *p*H 11.0 Buffer			
5.00		5.10	102.0
5.00		5.05	101.0
5.00		5.00	100.0
5.00		5.02	100.4
5.00		4.98	99.6
	Av.	5.03	100.6

[1] All buffers were prepared double strength.
[2] Clark and Lubs buffer: acid potassium phosphate and sodium hydroxide.
[3] Clark and Lubs buffer: boric acid, potassium chloride, and sodium hydroxide.

TABLE II.—PRECIPITATION OF VARIOUS B COMPLEX VITAMINS BY AMMONIA REINECKATE REAGENT AT DIFFERENT *p*H LEVELS

Compound,[a] Mg.	*p*H 7.0	8.0	9.0	10.0	11.0
Thiamine hydrochloride					
5	+	+	+	+	−
10	+	+	+	+	−
25	+	+	+	+	+
50	+	+	+	+	+
Pyridoxine hydrochloride					
5	+	−	−	−	−
10	+	−	−	−	−
25	+	−	−	−	−
50	+	−	−	−	−
Riboflavin					
5		−	−	−	−
10		−	−	−	−
25		−	−	−	−
50		−	−	−	−
Nicotinic acid					
5	+	−	−	−	−
10	+	−	−	−	−
25	+	−	−	−	−
50	+	−	−	−	−
Nicotinamide					
5	+	−	−	−	−
10	+	−	−	−	−
25	+	−	−	−	−
50	+	−	−	−	−
Pantothenic acid					
5	−	−	−	−	−
10	−	−	−	−	−
25	−	−	−	−	−
50	−	−	−	−	−
Choline chloride					
5	+	+	+	+	+
10	+	+	+	+	+
25	+	+	+	+	+
50	+	+	+	+	+

[a] Riboflavin at *p*H 7.0 was not sufficiently soluble to be investigated.

It was found that solutions of trisodium phosphate have a *p*H of approximately 11.6 and that a concentration of 5% trisodium phosphate produced immediate and complete destruction of a 50-mg. aliquot of thiamine hydrochloride at room temperature (20°), yielding no precipitate upon the immediate addition of ammonium reineckate reagent, as indicated in Table III.

TABLE III.—THE EFFECT OF TRISODIUM PHOSPHATE SOLUTIONS ON THE PRECIPITATION OF THIAMINE HYDROCHLORIDE BY AMMONIUM REINECKATE REAGENT

Trisodium Phosphate Solutions, %	*p*H	*p*H After Addition of 50 Mg. of Thiamine Hydrochloride	Ammonium Reineckate Reagent Added—At Once	After 15 Min.	After 30 Min.
1	11.6	10.9	+	+	−
2	11.6	11.0	+	−	−
5	11.6	11.4	−	−	−

It now became important to determine whether or not choline could be quantitatively recovered from 5% trisodium phosphate solution and, if so, whether or not it was stable for a period of time sufficient to make such determination feasible. Results of these investigations are given in Table IV.

TABLE IV.—THE EFFECT OF TIME UPON THE RECOVERY OF CHOLINE FROM 5% TRISODIUM PHOSPHATE SOLUTION

Choline Chloride, Mg.	Time in Min. Before Precipitation as Reineckate	Choline Chloride Recovered, %
5	0	102.2
5	30	101.0
5	60	100.0
5	120	100.5
5	180	99.7
5	240	101.2

It is apparent from Table IV that choline could be quantitatively determined in the alkaline 5% trisodium phosphate solution within limitations usually inherent in colorimetric or spectrometric methods. Also, it is apparent that choline is not readily decomposed in solutions of such high *p*H values on standing.

Using the following procedure, choline was determined in a mixture with other vitamins of the B complex at various concentrations equal to, or in excess of, the choline chloride present. Mixtures consisted of choline with other vitamins individually and together with all the factors of the B complex shown in Table V.

Procedure.—To 5 cc. of standard solution of choline chloride (containing 5 mg. of choline chloride), in a 50-ml. beaker, were added the other vitamins as indicated in Table V, so that the total volume of the solution did not exceed 10 cc. If less than 10 cc., sufficient distilled water was added to make the volume 10 cc. Ten cubic centimeters of 10% trisodium phosphate solution was then added and the mixture mixed well. To this solution 5 cc. of ammonium reineckate reagent was added and the mixture allowed to stand for thirty minutes. At the end of this time, the precipitate of choline reineckate was filtered off and determined as described in the procedure. Recovery of choline chloride from the various mixtures and concentrations is given in Table V.

DISCUSSION

Of the vitamins under investigation, only thiamine and choline produced precipitates with ammonium reineckate in alkaline solution. This similarity of reaction is due to the fact that both are quaternary ammonium salts. Thiamine is rapidly destroyed in alkaline solution, however, and this property may be utilized for the separation of the two compounds and the subsequent determination of choline. The alkaline decomposition products of thiamine do not yield precipitates with ammonium reineckate at *p*H 11.0 or higher, but do precipitate at lower *p*H values. For this reason the precipitation of choline reineckate should, in all instances, be carried out at *p*H 11.0 to 11.5. Trisodium phosphate, 5% solu-

TABLE V.—THE RECOVERY OF CHOLINE CHLORIDE FROM MIXTURES CONTAINING VARIOUS OTHER SYNTHETIC B COMPLEX VITAMINS AS INDICATED

Choline Chloride, Mg.	Thiamine Hydrochloride, Mg.	Riboflavin, Mg.	Pyridoxine Hydrochloride, Mg.	Nicotinic Acid, Mg.	Nicotinamide, Mg.	Pantothenic Acid, Mg.	Choline Chloride, % Recovery
5	5	..	..	..	..	..	101.3
5	10	..	..	..	..	..	100.9
5	25	..	..	..	..	..	99.6
5	50	..	..	..	..	..	99.4
5	..	5	..	..	..	..	98.9
5	..	10	..	..	..	..	101.7
5	..	25	..	..	..	..	101.0
5	..	50	..	..	..	..	99.8
5	..	..	5	..	..	..	99.3
5	..	..	10	..	..	..	100.3
5	..	..	25	..	..	..	101.0
5	..	..	50	..	..	..	99.7
5	..	..	..	5	..	..	98.8
5	..	..	..	10	..	..	99.5
5	..	..	..	25	..	..	98.8
5	..	..	..	50	..	..	101.1
5	..	..	..	..	5	..	98.9
5	..	..	..	..	10	..	99.1
5	..	..	..	..	25	..	102.0
5	..	..	..	..	50	..	101.1
5	..	..	..	..	..	5	99.0
5	..	..	..	..	..	10	99.6
5	..	..	..	..	..	25	98.9
5	..	..	..	..	..	50	101.6
5	5	5	5	5	5	5	101.4
5	10	10	10	10	10	10	100.6
5	25	25	25	25	25	25	99.4

tion, was found to be satisfactory in all combinations investigated in the scope of this work. Furthermore, the experimental work indicates that choline is relatively stable at *p*H values up to 11.5 as required for the separation.

Conditions of the method are rather severe, since the amounts of the B complex vitamins in all cases was as great or greater than the amount of choline present. Since the requirements for choline in all species of animals is considerably greater than for the vitamins investigated, it will be appreciated that, as a rule, the choline concentration of a product will usually be considerably in excess of the other vitamins present.

Choline determinations on a number of synthetic vitamin B complex mixtures, in both liquid and dry form, have been carried out with results generally within 2% of the mean values. It is significant that reproducible values could be obtained despite the high concentration of other materials present. Ascorbic acid in concentrations 20 times that of choline had no effect upon the determinations.

The method works equally well with other common choline salts such as the citrate and tartrate.

SUMMARY

A method for the determination of choline in the presence of significant amounts of synthetic vitamins of the B complex is described. The method is dependent upon the fact that pyridoxine, nicotinic acid, and nicotinamide, which ordinarily yield precipitates with ammonium reineckate in neutral or acid solution, do not precipitate with the reagent in alkaline solution. Thiamine, although yielding a precipitate in alkaline solution, is rapidly destroyed under certain conditions, so that choline alone is precipitated as the reineckate. The choline reineckate is filtered off, dissolved in acetone, and determined spectrophotometrically. Tables are given showing the effect of various buffers on the precipitation of the vitamins under study and the recovery of choline both alone and in admixtures with the various vitamins.

REFERENCES

(1) Jacobi, H. P., *et al.*, *J. Biol. Chem.*, **138**, 571(1941).
(2) Engel, R. W., *ibid.*, **144**, 761(1942).
(3) Handler, P., and Dann, W. J., *ibid.*, **146**, 357(1942).
(4) Glick, D., *ibid.*, **156**, 643(1944).
(5) Winzler, R. J., and Meserve, E. R., *ibid.*, **159**, 395 (1945).

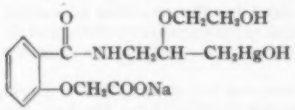
A New Mercurial Diuretic*

By E. BROWN ROBBINS and K. K. CHEN†

An investigation of a new mercurial shows it to have a diuretic potency in dogs comparable to mersalyl, U. S. P., but a lower acute toxicity in animals. It is less irritating to the mouse's skin than mersalyl. It may cause the formation of calcified masses in renal tubules of rats from repeated intramuscular injections of large doses. When injected intravenously in anesthetized cats, it lowers blood pressure and produces abnormal changes of electrocardiogram, with prompt recovery.

SINCE the discovery by Vogl (1) in 1919 of the diuretic action of certain organic mercurial compounds, the clinical use of these substances has increased yearly. All such compounds produce occasional toxic reactions when administered in therapeutic doses (2, 3). An improvement of mercurial diuretics could be achieved either by increasing the diuretic activity without increasing the toxicity or, conversely, by decreasing the toxicity without decreasing the diuretic activity. With this in mind, a series of organic mercurial compounds was tested for the two factors—diuretic activity and toxicity. Out of 111 compounds, one gave promise of a relative decrease of toxicity without sacrifice of activity. The results of an elaborate study on this product are presented in this communication.

Chemically, the compound is the sodium salt of *o*-(N-γ-hydroxymercuri-β-hydroxyethoxypropyl carbamyl)-phenoxyacetic acid, and bears our serial number 08879. It is similar to mersalyl, U. S. P., except for the substitution of the methoxy group in mersalyl by a hydroxyethoxy group, as shown by the following formula.



For sake of brevity, the compound will be frequently referred to by the number 08879. The substance is a white powder with no clear-cut melting point. Its sodium salt is readily soluble in water. In all experiments, fresh solutions were prepared to contain 2 parts by weight of 08879 (2–10 per cent) and 1 part by weight of theophylline (1–5 per cent). The addition of the latter facilitates absorption from subcutaneous or intramuscular injection, and reduces local reaction (4, 5). Whenever mersalyl was employed for comparison, it was also combined with theophylline, and in the same concentrations by weight.

EXPERIMENTAL

Diuretic Action.—Female dogs, fasted for twelve hours, were given physiological saline solution in the amount of 25 ml./Kg. by stomach tube. One hour later they were anesthetized with Amytal sodium (amobarbital sodium, Lilly) (60 mg./Kg. intraperitoneally). A Foley Bag (No. 12) inlying catheter was inserted into the urinary bladder, and the urine drained. The mercurial was administered intramuscularly one hour after the injection of the anesthetic. One-half hour after the injection of the mercurial, the urinary bladder was emptied, and fifteen-minute readings of urine output were recorded for a period of three hours. It had been found by repeated trials that the onset of the diuretic action occurred about one-half hour after the administration of the mercurial, and that the effect lasted for approximately three hours.

The doses of both 08879 and mersalyl were calculated in terms of mercury content, and comparisons were made at dose levels of 0.75, 1.5, and 3.0 mg. Hg/Kg. A cross-over comparison on 24 dogs was made with the dose of 1.5 mg. Hg/Kg., and the results are shown in Table I. In order to balance the effect of tolerance, 12 dogs were given 08879 first and mersalyl ten to fifteen days later, while in the other 12 dogs the order was reversed. The average urine output for 66 nontreated dogs was observed to be 2.4 ml./Kg. per three hours. Figures in Table I for urinary output under the influence of mersalyl and 08879 exceeded this figure, with five exceptions. The latter could be attributed to the poor susceptibility of these few animals. These results certainly prove the efficacy of either drug in increasing urinary output. The average (geometric) of 24 ratios in the last column indicates that the diuretic potency of 08879 is 86.8 ± 10.3% of that of mersalyl. The difference in activity, however, is statistically not significant. The results on 24 other dogs with doses of 0.75 and 3.0 mg. Hg/Kg., without cross-over, both favored 08879. From the over-all data, it appears that mersalyl and 08879 have a comparable onset, duration, and magnitude of diuresis.

In 19 experiments the sodium content of the urine was determined by means of the Beckman flame photometer, following various doses of 08879. There was an increase in sodium excretion which paralleled the increase in the urinary output in each experiment.

* Received November 2, 1950, from the Lilly Research Laboratories, Indianapolis 6, Ind.

† The authors are indebted to Drs. Paul N. Harris, Francis G. Henderson, and Mary A. Root, and to Robert C. Anderson and Richard M. Small, for their invaluable assistance in some of the experiments.

TABLE I.—THE DIURETIC ACTION OF COMPOUND 08879 AND OF MERSALYL IN DOGS. EACH DRUG ADMINISTERED IN DOSE OF 1.5 MG. HG/KG. INTRAMUSCULARLY

Dog No.	Mersalyl		Compound 08879		Ratio in %. 08879 / Mersalyl
	Date	Urine, Ml./Kg./3 Hr.	Date	Urine, Ml./Kg./3 Hr.	
1	4-20-50	9.60	5- 1-50	4.28	44.6
2	4-20-50	6.76	5- 1-50	3.64	53.8
3	4-21-50	5.82	5- 2-50	10.64	182.8
4	4-21-50	10.20	5- 2-50	11.00	107.8
5	4-24-50	2.78	5- 3-50	1.44	51.8
6	4-24-50	16.83	5- 3-50	10.16	60.4
7	4-25-50	5.00	5- 8-50	1.94	38.8
8	4-25-50	5.04	5- 8-50	4.24	84.1
9	4-26-50	5.79	5- 9-50	2.74	47.3
10	4-26-50	10.66	5- 9-50	6.68	62.7
11	4-27-50	4.66	5-10-50	2.72	58.4
12	4-27-50	6.14	5-10-50	6.26	101.9
13	5- 1-50	3.66	4-20-50	3.28	89.6
14	5- 1-50	4.48	4-20-50	8.44	188.4
15	5- 2-50	13.89	4-21-50	18.60	133.9
16	5- 2-50	6.16	4-21-50	7.54	122.4
17	5- 3-50	4.34	4-24-50	5.80	133.6
18	5- 3-50	3.90	4-24-50	22.06	565.6
19	5- 8-50	4.10	4-25-50	5.06	123.4
20	5- 8-50	11.78	4-25-50	5.36	45.5
21	5- 9-50	3.14	4-26-50	1.77	56.4
22	5- 9-50	12.94	4-26-50	13.42	103.7
23	5-10-50	1.76	4-27-50	1.90	108.0
24	5-10-50	21.45	4-27-50	10.94	51.0

Toxicity.—Acute toxicity studies were conducted in mice, rats, and rabbits, by intravenous and intramuscular injections with compound 08879 and mersalyl, both combined with theophylline. All animals were given a single dose, and observed for a week. Most deaths occurred within three days. The median lethal doses ± standard errors (LD_{50} ± S. E.) were computed according to Bliss' method (6). As noted in Table II, the new compound has a definite trend of being less toxic than mersalyl.

Subacute toxicity studies were made with 08879 in six weeks old albino rats. The drug was administered intramuscularly once a day, Monday through Friday, for a period of one month. Four dose levels were adopted—5.5, 11, 22, and 33, mg./Kg. (2.06, 4.13, 8.25, 12.38, mg. Hg/Kg., respectively). Groups of five animals were used for each dose level.

The three lowest doses of 08879 did not affect the rate of growth when compared with untreated control animals, while the largest dose inhibited growth slightly. Necropsy of the latter group, upon sacrifice, showed a few small irregular calcified masses in the renal tubules in the area of the cortico-medullary junction. Similar, but smaller and less numerous, calcified masses were found in the renal tubules of two rats each in the groups which were on daily doses of 11 and 22 mg./Kg. The animals receiving the smallest dose, namely 5.5 mg./Kg., were all normal. The calcified masses in the kidneys of the other groups of rats were probably the healed areas of mild injury resulting from repeated administration of 08879.

Irritation.—Solutions (10%) of 08879 and mersalyl, both combined with 5% theophylline, were tested on the shaved mouse skin for their irritant effect by means of the method of Lehman and coworkers (7). A volume of 0.02 ml. was injected intracutaneously on the abdomen. Ten mice each were employed for either mercurial. Twenty-four hours after injection, necrosis and ulceration occurred at the site of injection in one-half of the mersalyl animals. Compound 08879 produced no discernible irritation in any of the mice treated.

Circulation.—In view of the fact that the commonly used mercury diuretics cause electrocardiographic changes in experimental animals as reported by Lehman (8), work was initiated with 08879. Ten cats, anesthetized with Amytal sodium in the dose of 70 mg./Kg. intraperitoneally, were arranged for the recording of carotid blood pressure and electrocardiogram from lead II. The mercurial-theophylline solution was injected intravenously.

It was observed that doses of 08879, varying from 5 to 40 mg./Kg. (1.87 to 15 mg. Hg/Kg.), always resulted in fall of blood pressure amounting to 20 to 140 mm. Hg, with complete recovery in about fifteen minutes. Alterations of electrocardiograms occurred with doses of 10 to 20 mg./Kg., but none with a dose of 5 mg./Kg. The abnormal changes chiefly consisted of widening of QRS segment, reversal in the direction of T wave, atrioventricular dissociation, and short periods of ventricular fibrillation. The electrocardiographic pattern usually returned to normal within ten to thirty minutes, indicating the absence of any sustaining injury. There was a marked variation of individual susceptibility, for in three cats a dose of 40 mg./Kg. caused less deviations in the electrocardiogram than smaller doses in other animals. A few observations were made with a mersalyl-theophylline solution by intravenous injection in the same dosages. The drop in blood pressure and the derangement of the electrocardiogram were similar to those following the administration of 08879-theophylline.

TABLE II.—ACUTE TOXICITY OF COMPOUND 08879 AND MERSALYL

Animal	Route	08879 No. Used	08879 $LD_{50} \pm$ S. E. Mg./Kg.	Mersalyl No. Used	Mersalyl $LD_{50} \pm$ S. E., Mg./Kg.
Mouse	Intravenous	40	112.9 ± 10.8	40	72.6 ± 5.1
	Intramuscular	40	117.9 ± 8.1	40	97.4 ± 5.4
Rat	Intravenous	90	32.2 ± 2.8	90	17.7 ± 1.7
	Intramuscular	35	34.8 ± 4.4	20	24.1 ± 1.9
Rabbit	Intramuscular	24	26.2 ± 4.1	24	24.5 ± 2.1

DISCUSSION

The data presented above indicate that our new compound 08879 combined with theophylline has a degree of diuretic activity comparable to mersalyl. The former has an apparently lower acute toxicity and less irritation by injection into the skin. It may be possible, therefore, that 08879 when injected subcutaneously in man is less irritating than mersalyl. Like other mercurial diuretics, 08879 may cause injuries to the kidney from prolonged administration of large doses. This is evidenced by the occurrence of calcified masses in the renal tubules in treated rats. Temporary fall of blood pressure and abnormal changes in the electrocardiogram occur after intravenous injection of large doses of 08879. If this mercurial proves to be a useful diuretic clinically, the potential dangers of mercurials still will be present.

SUMMARY

An analog of mersalyl, the sodium salt of *o*-(N-γ-hydroxymercuri-β-hydroxyethoxypropyl carbamyl)-phenoxyacetic acid, has been investigated pharmacologically in combination with theophylline in the ratio of 2:1. When compared with mersalyl, U. S. P., also in combination with theophylline, in dogs, it has about the same diuretic potency, but a lower acute toxicity in mice and rats by intravenous and intramuscular injections. Prolonged daily administration intramuscularly in rats may result in formation of calcified masses in renal tubules. Intravenous injection of doses between 1.87 and 15 mg. Hg/Kg. in anesthetized cats produces a fall of arterial blood pressure and abnormal changes of electrocardiogram, with prompt recovery in both.

REFERENCES

(1) Vogl, A., *Am. Heart J.*, 39, 881(1950).
(2) Greenberg, D., and Feibush, J. S., *N. Y. State J. Med.*, 49, 2319(1949).
(3) Haas, H. T. A., *Pharmasie*, 2, 1(1947); through *Chem. Abstr.*, 42, 5996(1948).
(4) Goodman, L., and Gilman, A., "Pharmacological Basis of Therapeutics," Macmillan Co., New York, 1941, p. 639.
(5) Grollman, A., and Slaughter, D., "Cushny's Pharmacology and Therapeutics," Lea and Febiger, Philadelphia 1947, p. 143.
(6) Bliss, C. I., *Quart. J. Pharm. Pharmacol*, 11, 192(1938).
(7) Lehman, R. A., Taube, H., and King, E. E., *Proc. Soc. Expil. Biol. Med.*, 71, 1(1949).
(8) Lehman, R. A., *ibid.*, 64, 428(1947).

☆

American Association for the Advancement of Science—Pharmacy Subsection Meeting

The next meeting of the American Association for the Advancement of Science will be held in Philadelphia on December 26–31, 1951. The program of the Pharmacy Subsection of the Medical Sciences is being made up. Six sessions of this Subsection will be held. Members are invited to attend the meetings of the Section on Medical Sciences, Subsection on Medicine, and the Section on Chemistry.

Arrangements will be made for advance registration. Advance registrants will be sent a copy of the General Program before the meeting. See *Science* or *Scientific Monthly* in late August or September.

Authors who wish to present papers before the Subsection are requested to send in titles of papers *as soon as possible*. All titles for inclusion in the printed program must be in the hands of Glenn L. Jenkins, Chairman, Subsection Np, Purdue University, School of Pharmacy, Lafayette, Ind., by *September 15*.

Notes

A Note on the Preparation of the Tocopherol Blank in the Fox and Mueller Procedure for Vitamin A*

By D. G. CHAPMAN and J. A. CAMPBELL

Fox and Mueller (1) have recently devised a modification of the U. S. P. XIV method (2) for the estimation of vitamin A in the presence of tocopherols. It is based on the treatment of tocopherols with sulfuric acid originally suggested by Parker and McFarlane (3). The latter authors used 85% sulfuric acid, while Fox and Mueller prepared a tocopherol blank by washing the sample with 60% sulfuric acid. These authors claim that this treatment destroys the vitamin A without affecting the tocopherols. They point out that the conditions such as concentration of acid and time of reaction are critical.

In this laboratory, as part of a project on the separation of tocopherols (4), data have been obtained on the effect of different concentrations of sulfuric acid on type IV tocopherol concentrate.[1] It was felt that the results obtained might be of interest to those laboratories which would use the tocopherol blank in the estimation of vitamin A as described by Fox and Mueller (1). The data reported here confirm their findings that 60% sulfuric acid does not destroy tocopherols.

PROCEDURE

A 2% solution of type IV concentrate was prepared in petroleum ether. Ten cubic centimeters of this solution was placed in a 30-cc. glass-stoppered separatory funnel. To this was added 2 cc. of the sulfuric acid solution, and the funnel was inverted 10 times. The two phases were separated by centrifuging. One cubic centimeter of the petroleum ether phase was placed in a 50-cc. Erlenmeyer flask containing 15 cc. of ethanol and the tocopherols were titrated with ceric sulfate, using diphenylamine as the indicator. The tocopherol content was determined in this manner, both before and after washing with sulfuric acid.

RESULTS AND DISCUSSION

The effect of washing the 2% type IV solution with sulfuric acid solutions varying in concentration from 0 to 100% (v/v) is shown in Table I. The values reported are the averages of at least two determinations agreeing within 1%. It would appear that a 2% type IV solution in petroleum ether may be washed with water or a sulfuric acid-water solution containing up to 60% acid (v/v) without any loss of the tocopherols. When reagent grade sulfuric acid (undiluted) was used, some 84% of the tocopherols were destroyed. The technique used by the authors was very similar to that used by Fox and Mueller (1). It was found that it required fifteen seconds to invert a funnel 10 times, and hence even the time of contact was similar in both laboratories.

TABLE I.—THE EFFECT OF WASHING TYPE IV CONCENTRATE OF TOCOPHEROLS WITH SULFURIC ACID SOLUTIONS OF VARYING CONCENTRATION

Washing	Tocopherols Found, Mg./Gm.	Recovery, %
None	346	...
Water	346	100.0
H_2SO_4 (%, v/v)		
10	351	101.4
20	355	102.6
30	351	101.4
40	355	102.6
50	349	100.9
60	346	100.0
70	334	96.5
80	279	80.6
85	212	61.3
90	174	50.3
100	56	16.2

In subsequent work on the treatment of tocopherols with acid, duplicate concentrations (v/v) were found to give different specific gravity readings. Since sulfuric acid tends to take up water and hence change in concentration, it was found to be much more satisfactory to express the concentration of the acid in terms of specific gravity. As the concentration of the acid is critical, this practice eliminated one possible source of variation in the results obtained.

Other factors affecting the treatment of tocopherols with sulfuric acid are outlined elsewhere (5).

REFERENCES

(1) Fox, S. H., and Mueller, A., THIS JOURNAL, 34, 621 (1950).
(2) "United States Pharmacopeia," Fourteenth Revision, Mack Publishing Co., Easton, Pa., 1950.
(3) Parker, W. E., and McFarlane, W. D., *Can. J. Research*, B, 18, 405(1940).
(4) Chapman, D. G., and Campbell, J. A., Chemical Institute of Canada, Annual Conference, June, 1950.
(5) Chapman, D. G., Lichon, P., and Campbell, J. A., in press.

* Received February 16, 1951, from the Food and Drug Divisions, Department of National Health and Welfare, Ottawa, Canada.

[1] Obtained from Distillation Products Industries, Rochester, N. Y.

A Note on the Alkaloid Content of *Gloriosa Rothchildiana*, O'Brien*

By J. T. BRYAN and W. M. LAUTER

THE INCREASE in growing the ornamental plant *Gloriosa Rothchildiana*, O'Brien, a native of tropical Africa, in America suggested an investigation of its possible toxic constituents. Samples of tubers were obtained through the kindness of Mr. Wyndham Hayward, of Winter Park, Fla., and our investigation was carried out with this material.

It is known that *Gloriosa superba*, a close relative of *G. Rothchildiana*, contains colchicine as well as two other alkaloids, the constitution of which has not been determined (1, 2). Hence it was thought to be advisable to determine the presence or absence of colchicine, primarily because of its high toxicity.

Drying of the Plant Material.—The tubers were cut into thin, longitudinal slices and 83 Gm. of freshly prepared slices were dried at 60° for seventy-two hours.

The loss of moisture amounted to 40.05 Gm. or 47.9% of the undried tuber.

Ash Determination.—After converting the dried slices into a powder and screening through a No. 20 mesh sieve, total ash and acid-insoluble ash determinations were carried out according to official methods.

Total ash	
Sample 1	2.82%
Sample 2	2.80%
Sample 3	2.50%
Average	2.70%
Acid-insoluble ash	None

Extraction.—Two hundred grams of dried material (see above), after powdering and screening through a No. 20 mesh, was extracted in a Soxhlet apparatus. We followed the procedure as described by Clewer, *et al.* (1), for *Gloriosa superba*. Ethanol (450 cc.) was used. The extraction was carried out for fourteen hours. The alcoholic solution became dark brown in color and a slight gummy precipitate could be observed.

Upon nearly complete removal of the ethanol by evaporation, a brown, viscous residue remained.

Isolation of Colchicine.—Upon cooling, two distinct types of crystalline material could be discerned in the residue.

A qualitative test with Mayer's reagent was positive. An alcoholic solution produced a red color wth ferric chloride. This behavior made us proceed on the assumption that colchicine would be present.

The total residue was then transferred to a steam-distillation flask and steam-distilled until the distillate was only slightly cloudy. The distillate was then saturated with NaCl, but failed to set free any oily fractions.

Since Clewer, *et al.* (1), had reported the presence of furfural in *G. superba*, we tested the distillate for furfural as described by Guenther (3). This test also was positive.

The residue and water remaining in the steam-distillation flask were separated from each other by filtration. The aqueous portion was then extracted with ether until no more positive tests could be obtained with Valser's reagent in the ether portion. This would remove other alkaloidal constituents, but not the colchicine.

The aqueous portion was then extracted with chloroform until the chloroform no longer gave an alkaloidal reaction with Valser's reagent. This should have removed the colchicine from the aqueous solution.

The chloroform was evaporated and a yellow semi-solid residue remained. After cooling to −10° this residue was extracted with anhydrous ether. The resinous material now became solid. After decantation of three portions of anhydrous ether, 0.42 Gm. of yellow crystalline material was obtained. Attempts to purify the material by dissolving it in ethyl acetate and reprecipitation with anhydrous ether gave 0.092 Gm. of a yellow precipitate which did not melt at 275°, and upon combustion gave off a caramel-like odor.

Hence, the ethyl acetate-anhydrous ether solution was evaporated and the yellow crystalline residue redissolved in water.

The aqueous solution then was again extracted with chloroform and this solvent removed by evaporation. The residue was extracted again several times with anhydrous ether and the yellow crystals remaining after decantation of the ether were dried over $CaCl_2$ *in vacuo*.

The yield was 85 mg. The melting point was 143–145°. A sample of U. S. P. colchicine melted at 143–145°. A mixed melting point showed that the material isolated was colchicine.

The identification tests as described in the U. S. P. XIII were applied and established the fact that this isolated material was indeed colchicine.

DISCUSSION

The presence of colchicine in the widely cultivated ornamental plant *Gloriosa Rothchildiana* has been proved. Only 0.043% of the pure alkaloid could be isolated from the dried tubers by the method we employed, but probably larger quantities are actually present.

It is also quite reasonable to assume that other alkaloids, possibly of the types as described by earlier investigations of *G. superba*, may be present. However, since their toxicities are at present little understood, no attempt has been made to isolate them at this time. We assume them to be present in the ether fractions described in this investigation.

However, the presence of the highly toxic alkaloid colchicine in *G. Rothchildiana* seems to indicate the rather poisonous nature of this garden plant.

SUMMARY

Colchicine has been shown to be present in the tubers of *Gloriosa Rothchildiana*.

REFERENCES

(1) Clewer, H. W. B., Green, S. J., and Tutin, F., *J. Chem. Soc.*, **107**, 835(1915).
(2) Parthasarathy, N., *Current Sci. (India)*, **10**, 446(1941).
(3) Guenther, E., "The Essential Oils," 1st ed., Vol. I. D. Van Nostrand Company, Inc., New York, 1948, p. 313.

* Received January 26, 1951, from the College of Pharmacy, University of Florida, Gainesville.

Preliminary Report of Lysis of Mouse Sarcoma 37 by a Component Isolated from Resin of Podophyllum*

By MURIEL R. LORAN† and EARL P. GUTH‡

On October 2, 1949, a successful separation of a new component of resin of podophyllum was made by a special procedure. On July 6, 1950, the first of numerous animal experiments were begun to test this material pharmacologically. In a preliminary experiment three mice—one of which had a spontaneous tumor—were injected with the substance. One of the three died and two survived, including the one with a spontaneous tumor. The two surviving mice were very sick, but recovered. Upon recovery, no trace of tumor could be found on the one mouse.

On July 10, 1950, another preliminary toxicity experiment was run using ten mice, one of which again had a spontaneous tumor. The same phenomenon occurred.

After these preliminary experiments, regular LD_{50} determinations were made with the material, and toxicity and gross effects on normal Swiss albino mice determined.

After extensive work on the chemistry and pharmacology of the material, the possibility that this substance would lyse a mouse tumor was investigated. Various references in the literature had indicated the possibility that the resin would be capable of lysing tumors, although most of the studies were carried out *in vitro* and were not very extensive. All previous studies were made with the crude resin of podophyllum.

On March 1, 1951, a preliminary test was run on one mouse having a transplanted 37 tumor, resulting in complete lysis of the tumor. The experiment was repeated on March 8, 1951, with four mice bearing transplants and with two controls. The result was again lysis of the tumor. All of the mice survived the treatment.

It was then decided that it would be propitious to run an entire screening procedure of the substance with male, white, Swiss albino mice bearing 37 transplants. Both massive dose and cumulative dose tests, for tumor lysis and tumor inhibition were administered. Total controls and experimental animals used numbered 400. This experiment was begun on March 26, 1951.

The result of this experiment showed that the substance isolated from resin of podophyllum will lyse sarcoma 37 mouse tumor. At least 50% of the animals had no viable tumor twenty-four hours after the first injection, and 100% of the tumors showed varying degrees of lysis.

This substance has a low toxicity. Histological examination revealed inflammation of kidney, liver, and intestine. Inflammation was observed specifically on the intestine and was not severe.

Continued research is in progress.

* Received April 16, 1951, from the Research Laboratories, The Ohio State University, College of Pharmacy.

† Graduate Student, The Ohio State University, College of Pharmacy.

‡ Professor of Pharmacy, The Ohio State University, College of Pharmacy.

☆

BOOKS RECEIVED

Principles and Practice of Bacteriology. By Arthur H. Bryan and Charles G. Bryan. Barnes and Noble, Inc., New York, 1951. xv + 410 pp. Illustrated. 13.5 x 21 cm. Price $1.75.

The Biochemistry of Inositol. By Edward R. Weidlein, Jr. Bibliographic Series Bulletin No. 6. Mellon Institute, Pittsburgh, Pa., 1951. v + 53 pp. 15.5 x 23 cm. Price: free.

Methods of Vitamin Assay. 2nd ed. Prepared and edited by the Association of Vitamin Chemists, Inc. Interscience Publishers, Inc., New York, 1951. xviii + 301 pp. 16 x 23.7 cm. Price $5.50.

Qualitative Arzneimittelanalyse. By Hans Mühlemann and Adolf Bürgin. Ernst Reinhardt Verlag AG., Basel, Switzerland, 1951. 285 pp. 13 x 21 cm.

Recent Advances in Chemotherapy. 3rd ed. Vol. II. By G. M. Findlay. The Blakiston Company, Philadelphia, 1951. xi + 597 pp. 14 × 20.8 cm. Price $7.50.

Progress in Chromatography, 1938–1947. By L. Zechmeister. John Wiley and Sons, Inc., New York, 1951. xviii + 308 pp. Illustrated. 14.5 x 22.5 cm. Price $8.

Soybeans and Soybean Products. Vol. II. Edited by Klare S. Markley. Interscience Publishers, Inc., New York, 1951. xvii + 604 pp. Illustrated. 16 x 23.5 cm. Price $11.

The Amino Acid Composition of Proteins and Foods. 2nd ed. By Richard J. Block and Diana Bolling. Charles C Thomas, Springfield, Ill., 1951. xxxviii + 576 pp. Illustrated. 16 x 25.5 cm. Price $10.50.

Histamine Antagonists. By Frederick Leonard and Charles P. Huttrer. National Research Council, Washington, D. C., 1950. 122 pp. 17.5 x 25 cm. Price $1.50.

Maize in the Great Herbals. By John J. Finan. The Chronica Botanica Co., Waltham, Mass.; Stechert-Hafner, Inc., New York City. xiv + 43 pp. Illustrated. 17.5 x 25.5 cm. Price $3.

Book Reviews

The Polarographic Method of Analysis. By OTTO H. MÜLLER. Chemical Education Publishing Co., Easton, Pa., 1951. xii + 209 pp. Illustrated. 13.5 x 21 cm. Price $3.50.

Chemists who have been interested in using the polarographic method of analysis will recall that the author of this book published a series of five articles in 1941 in the *Journal of Chemical Education*, which were eventually reprinted and bound together to constitute the first edition of *Polarographic Method of Analysis*.

The purpose of the first edition was, according to the author, "to present a simple account of polarography in a form which can be used by teachers and students of physical chemistry as well as in advanced courses of analytical chemistry." The aim of the second edition is essentially the same, but this edition has been thoroughly revised, with the incorporation of recent developments to polarography.

The book is divided into eight chapters, an appendix, a list of general polarographic references, a list of experiments, and an index. In the first chapter a brief review of electro-analytical methods is given, mainly to emphasize the fact that Professor Jaroslav Heyrovský, of the Charles' University, Prague, developed an electrochemical method of analysis quite distinct in its principles from others.

In Chapter II the essential apparatus required in polarography is described. The fundamentals of quantitative analysis, included in Chapter III, has been expanded by adding sections on the kinetic and catalytic currents. More emphasis on reactions in poorly buffered solutions has been incorporated in Chapter IV on the fundamentals of qualitative analysis. In Chapter V polarimetry has been treated separately. A brief description of some recent developments such as differential, derivative, and oscillographic polarography has been included in Chapter VI. The applications, discussed in Chapter VII, have been considerably increased in scope. Chapter VIII is entirely new and is devoted to suggestions for practical polarography, with special attention given to the characterization and selection of capillaries for the dropping mercury electrode.

The typical experiments listed serve to illustrate the points discussed in the preceding chapters. They can be carried out with very simple equipment ordinarily available in modern student laboratories of physical and analytical chemistry.

Polarographic methods applied to analytical pharmaceutical problems have been quite numerous during the past ten years. It seems safe to predict that the polarographic method may continue in the future to be applied to additional analytical problems of a pharmaceutical nature. There can be no doubt but that this second edition of *The Polarographic Method of Analysis* will aid anyone interested in learning the fundamentals of polarography.

Qualitative Arzneimittelanalyse. By HANS MÜHLEMANN and ADOLF BÜRGIN. Ernst Reinhardt Verlag AG., Basel, Switzerland, 1951. 285 pp. 13 x 21 cm.

This book, according to the authors, is intended mainly for the professional pharmacist and the student of pharmacy. Its purpose is to enable the user to carry out simple analytical procedures required in the practice of pharmacy. The tests outlined and the procedures described are all designed so as not to require the use of special and expensive equipment.

The book is divided into three main parts. The first part covers general methods of separation, the second part describes methods for the isolation of drugs from the dosage forms in which they are usually employed, and the third part describes characteristics and methods of identification of drugs previously separated and purified.

The book probably offers little of interest to pharmaceutical chemists in the United States. A large proportion of the drugs for which identification tests are given are included in either the United States Pharmacopeia or the National Formulary, thus making a supplementary book limited to simple identification tests unnecessary.

Salben, Puder, Externa (Die äusseren Heilmittel der Medizin). 3rd ed. By HERMANN v. CZETSCH-LINDENWALD and FRIEDRICH SCHMIDT-LA BAUME. Springer-Verlag, Berlin, 1950. xi + 492 pp. Illustrated. 16 x 23.8 cm. Price DM 36.

The main object of this book is to present information on the use of ointments. In the general part are presented an historical review and information on the basic materials of ointments used in dermatology. In this section will be found a classification of ointments according to their constituents. The second part of the book is devoted to therapeutics involving the use of ointments and comparable external preparations. In the third part various powders and powder bases are discussed. Of especial interest are the suggestions made for the choice of a powder or an ointment in the treatment of diseases of the skin. In the final chapter of the book industrial problems in protective skin preparations and methods of protecting the skin of workers from industrial poisoning are presented. The book is provided with a useful author and subject index.

Annual Report on Essential Oils, Aromatic Chemicals, and Related Materials. 1947–1948. Schimmel and Co., Inc., New York, 1951. 125 pp. Illustrated. 15 x 23 cm. Price in U. S. and Canada, $3; Elsewhere, $3.25.

The latest Schimmel Report has recently made its appearance covering two years (1947–1948), instead of one, as has been customary in previous editions. This year's edition offers the usual record of

scientific and commercial developments, research, and other information in the field of essential oils and aromatic chemicals during the period covered by the report. The book is organized along the same general lines as the 1945 and 1946 editions, following an alphabetical arrangement which has been found to be helpful to those using the work for references.

One interesting innovation is the inclusion of a brief foreward to the Essential Oil section drawing attention to a few of the more outstanding developments covered. A paragraph of similar intent opens the section dealing with research on Aromatics. The illustrations included in the 1947–1948 report are varied, well chosen, and unusually clearly reproduced, thus lending additional interest to the volume.

A study of the 1947–1948 chemical report leaves one with the opinion that considerable interest in essential oils and essential oil chemistry still exists, despite the wide use of synthetic aromatic chemicals in the fabrication of artificial flavors and perfumes. *The Annual Report on Essential Oils, Aromatic Chemicals and Related Materials* is of the same high quality and thoroughness as its predecessors. It constitutes worth-while reading for anyone with a serious interest in this field, and makes fascinating reading for anyone having an academic or casual interest in essential oils and related materials.

Practical Microscopy. 2nd ed. By L. C. MARTIN and B. K. JOHNSON. Chemical Publishing Co., Inc., Brooklyn, N. Y., 1950. 124 pp. Illustrated. 12.5 x 19 cm. Price $2.50.

Practical Microscopy presents, in a style interesting to read and easy to understand, information necessary to apply the technique of modern microscopy in practice. The optical principles involved in the microscope are explained in an unusually clear and concise manner. A study of these principles will aid anyone interested in microscopy in using the microscope so as to obtain the best possible results. The construction of the more commonly used microscope accessories is described and directions for their use quite clearly explained.

Chapters on ultraviolet microscopy and the electron microscope are included. The chapters on photomicrography, the preparation of specimens for the microscope, polarized light and the microscope, and on the interpretation of the image in the microscope make interesting reading for anyone interested in the principles of microscopy. The authors have succeeded in their aim to present a book of a practical nature.

Recent Advances in Chemotherapy. 3rd ed. Vol. II. *Malaria*. By G. M. FINDLAY. The Blakiston Company, Philadelphia, 1951. xi + 597 pp. 14 x 20.8 cm. Price $7.50.

The development and availability of chemotherapeutic agents, together with vector control, offer hope for the more than 700,000,000 persons infected with malarial plasmodia and for the new millions infected annually throughout the world. The author has collected and correlated the results of investigations on the chemotherapy of malaria reported in all countries. The book is replete with tabulations of data from many published reports.

A chapter is devoted to procedures for testing the antimalarial activity of chemotherapeutic agents. The pharmacology and the toxic reactions of antimalarial drugs are reviewed. Chapters are devoted to the biology of malaria in relation to chemotherapy, drug resistance and immunity, and the mode of action of antimalarial drugs.

The development of antimalarial chemotherapeutic agents is traced with the liberal use of structural formulas. These compounds are discussed under the following classifications: (I) The Cinchona Alkaloids and Their Modifications; (II) Pamaquin and Other 8-Aminoquinolines; (III) Mepacrine; (IV) Derivatives of 4-Aminoquinoline; (V) Acridone Derivatives; (VI) Pyridoacridines and Benzacridines; (VII) α-Aminocresols and *o*-cresols; (VIII) Cinnoline Derivatives; (IX) Carbamates; (X) Thio- and Dithio-Compounds; (XI) Guanidines; (XII) Proguanil (Paludrine); (XIII) Other Pyrimidine Derivatives; (XIV) Quinazolines.

The effects of molecular rearrangement and substituent changes upon the therapeutic index of compounds are discussed. It has so far proved impossible to correlate chemical constitution and antimalarial action.

The book is written in a lucid style and makes fascinating reading. The examination of fingernails under ultraviolet light to detect evasion of suppressive mepacrine (Atabrine) therapy is mentioned. The drug is deposited in the nails, which give a green fluorescence under ultraviolet light. Valuable features of the book are the complete author and subject indexes containing references to compounds, chemical reactions, and formulas. A bibliography is given at the end of each chapter.

This volume is a comprehensive reference compilation in its field. The author, who is the editor of *Abstracts of World Medicine* and *Abstracts of World Surgery, Gynecology and Obstetrics*, has apparently covered the literature completely. Dr. Findlay's book is recommended to anyone interested in the chemistry and the therapeutic use of antimalarial drugs.

Synthetische Methoden der Organischen Chemie. Vol. 4. By W. THEILHEIMER. Interscience Publishers, Inc., New York, 1950. xi + 360 pp. 16 x 23.5 cm. Price $9.25.

This book constitutes the fourth volume in a series designed to collect and make available organic synthetic methods published during the preceding year. Actually it consists of abstracts from papers published in 1947 and 1948, with some supplementary references from 1949. Titles relating to reactions from preceding volumes of the series have been included only when new references have been added. A continuation of this series has been made possible by grants from the chemical industry. It is planned that the series will be utilized in the future to record continuous new methods for the synthesis of organic compounds, improvements of known methods, and also older proved methods that are now widely scattered in original papers and periodicals. The abstracts presented in this volume, as in previous volumes, are limited to the information needed for an approval of the abstractability of a desired synthesis.

The book is provided with an alphabetical index limited to this volume. It is planned to include a cumulative index in Volume V and to publish that volume in English.

How many of these POTASSIUM THIOCYANATE features are important to you?

Check for Yourself ✓

- ✓ It is an all-synthetic product.
- ✓ Crystals* are colorless, transparent, and odorless.
- ✓ It is readily soluble.
- ✓ You have uniform quality and scheduled availability for Baker controls all raw materials.
- ✓ Precise process control insures high chemical purity.
- ✓ Containers are fiber, moisture-resistant drums polyethylene lined with friction cover tops to safeguard against atmospheric moisture.
- ✓ It is packaged in convenient 225, 100, 50 and 25 pound fiber drums.

*Picks up moisture. Should be kept in tightly closed containers.

You, too, will want to discover the superiority of Baker Potassium Thiocyanate in C.P. and N.F. Crystals.

Drug manufacturers, one after another, investigated its seven features—made comparative tests—and decided that this all-synthetic Potassium Thiocyanate had qualities that would, in turn, give their products superiority.

Users of Baker Potassium Thiocyanate, N.F. appreciate the assay of 99% plus and the unusually low indices of arsenic and heavy metals. Baker C.P. grade is chosen particularly for its low iron, chloride and sulfate content. When compounding medicinals related to arterial hypertension, these features of Baker Sodium and Potassium Thiocyanates are particularly important.

Check every one of the seven Baker features. Determine for yourself how many of these features are important to you. Then ask for samples and prices.

The comparative tests will be your best guide to value.

Address: J. T. Baker Chemical Co., *Executive Offices and Plant,* Phillipsburg, New Jersey

Baker CHEMICAL

Baker Chemicals

REAGENT • FINE • INDUSTRIAL

Now... a PYREX Beaker with a *Handle*

Here is a new feature—one which many have asked for—to help make your work easier and safer—a 3000 ml. beaker with a sturdy glass handle. Because you can grip it firmly, the danger of dropping a beaker filled with hot liquid is eliminated. The glass handle is attached with a stainless steel band which also acts as a protective collar. Handles and bands may be used interchangeably.

Made of PYREX brand glass No. 7740, the beaker can be used for a wide range of service because this glass is *the* ideally balanced glass—a glass which is both chemically stable and resistant to thermal and physical shock. To prevent chipping, the top rim of the beaker is heavily beaded.

Your laboratory dealer stocks these beakers for you. Give him a call today.

CORNING GLASS WORKS PYREX **CORNING, N. Y.**

Corning means research in Glass

1851 • 100 Years of Making Glass Better and More Useful • 1951